Effects of calcium supplementation and lactation on iron status¹⁻⁴

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ABSTRACT Calcium has been shown to inhibit iron absorption. The consequences of chronic calcium supplementation on iron status are unclear, however. As part of a randomized calcium-supplementation trial in lactating and nonlactating women in the postpartum period, we determined whether long-term calcium supplementation and lactation status affected iron stores as measured by serum ferritin concentrations. Subjects (95 lactating and 92 nonlactating) were enrolled at ≈6 mo postpartum and then randomly assigned to receive either 500 mg Ca as calcium carbonate or a placebo twice daily with meals for 6 mo. Lactating women weaned their infants ≈2 mo after enrollment (ie, ≈8 mo postpartum). Calcium supplementation had no effect on serum ferritin concentrations. At the end of the study, geometric mean serum ferritin concentrations were 28.4 μg/L in the calcium-supplemented group and 27.5 μg/L in the placebo group (P > 0.5). Lactation status was significantly related to serum ferritin concentrations. At baseline, serum ferritin concentrations were higher in lactating women than in nonlactating women (47.7 compared with 31.5 μg/L, P < 0.001). In lactating women, serum ferritin concentrations decreased by a mean of 17 μg/L after weaning. By 12 mo postpartum, mean serum ferritin concentrations in women who were previously lactating were not significantly higher than those of nonlactating women (30.5 compared with 25.5 μg/L). These findings provide reassurance that long-term calcium supplementation does not impair iron stores. Furthermore, lactation status should be considered when assessing iron nutriture of women and determinants of iron status in populations. Am J Clin Nutr 1998;67:1244–9.

KEY WORDS Lactation, weaning, postpartum period, ferritin, iron stores, iron status, calcium supplementation, calcium carbonate, randomized trial, women

INTRODUCTION

Consumption of calcium supplements has become more common as the health and economic consequences of osteoporosis have come to be better appreciated (1). However, calcium, given as a supplement or in the form of dairy products, may reduce both heme- and nonheme-iron absorption by 40–60% (2–6). Calcium appears to have the largest effect on iron absorption when consumed with an iron-containing meal and no effect if consumed ≥2 h after the meal (7). Consumption of calcium supplements with meals is recommended to enhance the bioavailability of calcium in the supplement (8). Iron status may, therefore, be compromised by long-term use of calcium supplements or intake of foods high in calcium, particularly in women of reproductive age in whom iron deficiency is common (9).

The nutritional consequences of long-term calcium supplementation in free-living individuals has received little attention. Yan et al (10) found no effect on serum ferritin concentrations of giving lactating Gambian women (n = 60) a daily dose of 1 g Ca as calcium carbonate. However, the supplement was given between meals so the opportunity for inhibition of iron absorption was minimized. Sokoll and Dawson-Hughes (11) found no effect on serum ferritin concentrations when healthy premenopausal women (n = 109) were given 250 mg Ca as calcium carbonate twice daily with meals for 12 wk.

As part of a randomized calcium-supplementation trial in lactating and nonlactating women in the postpartum period, we determined whether long-term supplementation with 500 mg Ca taken twice daily with meals affected iron stores as measured by serum ferritin concentrations. Because of the nature of the study population, we also describe the effects of lactation and weaning on maternal iron stores. To our knowledge, no reports in the literature have specifically evaluated the effects of lactation and weaning on maternal iron stores.

SUBJECTS AND METHODS

Study subjects participated in a randomized calcium-supplementation trial in women that was designed to evaluate the effects of calcium supplementation on changes in bone density postpartum (12, 13). Women were recruited from postpartum hospital wards, through newspaper advertisements, and by word of mouth. Iron status was assessed only in subjects who participated in the...
study between ≈6 and 12 mo postpartum. These women (95 lactating and 92 nonlactating) were enrolled in the study an average of 5.6 ± 0.8 mo postpartum. Lactating women were breast-feeding 5.4 ± 1.1 times daily and providing no more than one supplemental formula feed per day (111 ± 90 mL) at the time of enrollment. Lactating women weaned their infants from breast milk in the first 2 mo (7 ± 4 wk) after enrollment (ie, ≈8 mo postpartum). We refer to this group as lactating or previously lactating to reflect their status at the time measurements were obtained. Nonlactating women had either fed their infants formula exclusively from birth (n = 86) or had breast-fed for ≤2 wk (n = 6).

Four study groups were created by randomly assigning half of the women in each feeding group (lactating and nonlactating) to receive either 1 g Ca/d as calcium carbonate (Os-Cal; Marion Merrell Dow, Kansas City, MO) or a placebo containing lactose. Both subjects and study personnel were blinded to calcium treatment assignment. The supplements were provided as two tablets containing 500 mg Ca each, and subjects were instructed to consume the supplement at two separate meals to facilitate absorption of the calcium. There was no measure of whether the supplements were consumed with meals. The calcium supplement and placebo were consumed for 6 mo. Compliance with supplementation was determined by pill counts performed every 3 mo. All women in the study had low to moderate habitual calcium intakes (≤800 mg/d) as determined by a food-frequency questionnaire (14) as part of the main trial design. Subjects were also given a standard multivitamin containing 60 mg vitamin C to consume daily throughout the study and were asked to discontinue use of all other vitamin and mineral supplements before enrollment.

Blood samples were obtained at enrollment (ie, ≈6 mo postpartum) and 12 and 24 wk after calcium supplementation began (ie, 9 and 12 mo postpartum). Hemoglobin, hematocrit, and mean corpuscular volume (MCV) were measured immediately in the clinical laboratory at Children’s Hospital Medical Center. Serum samples were frozen at −70 °C for ferritin and C-reactive protein (CRP) determinations so that all samples for a given subject could be included in the same assay. Serum ferritin was measured by a double-antibody radioimmunoassay (Diagnostic Products Corporation, Los Angeles). The intraassay and interassay CV's in our laboratory for the low control (35 μg ferritin/L) were 10.5% and 17.7% and for the high control (189 μg ferritin/L) were 4.0% and 5.3%, respectively. Subjects were classified as having depleted iron stores (ferritin < 12 μg/L), microcytosis (MCV < 80 fl), or anemia (hemoglobin < 120 g/L) (16). CRP was measured by a latex agglutination test (IMMUNEX CRP; Wampole Laboratories, Cranbury, NJ). This test provided qualitative information: samples were either positive or negative for CRP. Positive and negative controls were run with each sample. CRP was measured to control for the potential effects of inflammation and infection on serum ferritin concentrations because ferritin and CRP are both increased in these situations (15, 16).

Subjects kept two 3-d food records during the study, during the 11th and 23rd study weeks. Nutrient intake was estimated with the NUTRITION DATA SYSTEM (University of Minnesota Coordinating Center, Minneapolis). Information on iron supplement use before enrollment in the study was recorded. Subjects were questioned about brand names, iron content, frequency, and duration of use of multivitamins, prenatal vitamins, and iron supplements.

The study was approved by the Institutional Review Board at Children’s Hospital Medical Center and all women provided written, informed consent to participate. Of the 187 women who enrolled, 158 completed the study successfully. The reasons for subjects being dropped from the study included loss of interest in the study (n = 1), prolonged illness or chronic medication use (n = 5), iron supplementation for anemia (n = 1 nonlactating woman in the placebo group), use of hormonal contraceptives (n = 1), pregnancy (n = 8), not weaning infant within 3 mo (n = 9), inability to swallow pills (n = 1), and relocation of subject or inability to contact subject (n = 3).

The effect of calcium supplementation on iron status was determined with repeated-measures analysis of variance, in which the primary outcome variable was serum ferritin concentration. Similar analyses were performed for the secondary outcomes of hemoglobin concentration, MCV, and hematocrit. The distribution of serum ferritin concentrations was skewed upward so the square roots of the ferritin values were used in all analyses. Potential confounders considered in the analyses included lactation group (lactating or nonlactating), dietary iron and vitamin C intakes during the study, and duration of iron supplement use before enrollment. Interaction terms with calcium-supplementation group (supplement or placebo) and baseline iron status and lactation group were also tested.

The effects of lactation on iron status were evaluated in two ways. First, cross-sectional analyses were performed by comparing mean serum ferritin concentrations at baseline (ie, ≈6 mo postpartum) between lactating and nonlactating women. Analysis of covariance was used to adjust for differences in the duration of postpartum use of iron-containing supplements between groups. Second, we compared the changes in serum ferritin concentrations that occurred in lactating women coincident with weaning with the changes observed in nonlactating women over the same time interval by using analysis of covariance. These changes were calculated as the differences between serum ferritin concentrations at baseline (≈6 mo postpartum), when lactating women were still fully breast-feeding, and serum ferritin concentrations at 9 and 12 mo postpartum (all lactating women had completely ceased breast-feeding by 9 mo postpartum). Duration of postpartum iron supplement use, calcium supplement group, and dietary iron and vitamin C intakes were included as covariates in the analyses.

All analyses were performed with and without subjects with positive CRP results. Because the results were not appreciably altered when subjects with positive CRP results were excluded, only results for the whole sample are presented. Statistical analyses were performed with JMP statistical software (version 3; SAS Institute Inc, Cary, NC).

RESULTS

There were no significant differences among groups in age, parity, dietary iron intake, or vitamin C intake during the study (Table 1). At enrollment, only 14.5% of lactating women had resumed menses whereas 98.8% of nonlactating women had these proportions did not differ by calcium supplement group. All but one woman in the lactating group had resumed menses by the end of the study (ie, ≈12 mo postpartum).

By design, all women had taken prenatal vitamin and mineral supplements during pregnancy that contained 60–90 mg Fe. In addition to prenatal vitamin and mineral supplements, 25% (39/158) of women took supplemental iron during pregnancy for a mean (±SD) of 6.4 ± 2.6 mo. There was variable use of iron-
containing supplements postpartum before enrollment into the study: 78% reported taking prenatal vitamin and mineral supplements or other multivitamins containing iron postpartum and 15% reported taking iron supplements postpartum. Almost all subjects who took supplements reported that they took them daily. Because many women could not remember the brand name or the iron content of the supplement they consumed, subjects were classified according to the maximum duration of iron or iron-containing supplement use. The tertile distributions of duration of iron-containing supplement use by lactation and calcium supplement groups are given in Table 1. Lactating women were more likely than nonlactating women to take iron or iron-containing supplements postpartum (92% compared with 71%, respectively, \( P < 0.0001 \)) and took them longer.

Overall, 60 of 457 (13%) serum samples tested positive for CRP, and there were no differences in the proportion testing positive across the four study groups at any sampling time (data not shown). There were insufficient serum samples available to perform the CRP test on 17 samples (3.6% of the total). One subject was excluded from all subsequent statistical analyses because her initial serum ferritin concentration was 465 μg/L, which was 14.5 SDs above the group mean. The mean serum ferritin concentration was higher for samples that tested positive for CRP than for those that tested negative (\( P < 0.01 \)): the geometric means (−1 SD, +1 SD) were 40.3 (14.4, 79.2) and 31.9 (13.2, 59.1) μg/L, respectively. Hemoglobin concentration, MCV, and hematocrit did not differ between CRP-positive and CRP-negative samples (\( P \geq 0.3 \)).

**Effects of calcium supplementation on iron status**

The mean serum ferritin concentrations of each of the four study groups during the study are shown in Figure 1. At baseline, serum ferritin concentrations differed between the lactating and nonlactating groups (see the next section), but there were no significant differences between the calcium-supplemented and placebo groups within each lactation group or averaged across lactation groups (\( P > 0.40 \)). Calcium supplementation did not significantly affect serum ferritin concentrations. The supplement group × time interaction term, which formally tests the effect of calcium supplementation, was not significant (\( P = 0.8 \)), and the three-way interaction term (lactation group × supplement group × time) also was not significant (\( P = 0.16 \)). At the end of the study (≈12 mo postpartum), the geometric mean serum ferritin concentrations (−1 SD, +1 SD) averaged across lactation groups were 28.4 (12.3, 51.0) μg/L in the calcium-supplemented women and 27.5 (9.3, 55.0) μg/L in the placebo-supplemented women (\( P = 0.7 \)). The results did not change \( I \) when the outcome variable was expressed as change from baseline (eg, final − initial serum ferritin concentration) and dietary iron and vitamin C intakes and iron supplementation use postpartum were included as covariates in the analyses, or \( 2 \) when the sample was restricted to the 142 women whose baseline serum ferritin concentration was ≥12 μg/L. Furthermore, the proportion of subjects with depleted iron stores (serum ferritin < 12 μg/L) at the end of study did not differ between calcium-supplemented (15.6%) and placebo (21.2%) groups (\( P = 0.4 \)).

Mean hemoglobin concentrations, MCV, and hematocrit by lactation and calcium-supplementation groups are given in Table 2. As for serum ferritin, there was no effect of calcium supplementation on these indicators of iron status (\( P \geq 0.3 \)).

**Effects of lactation on iron status**

At baseline, serum ferritin concentrations were significantly higher in lactating than in nonlactating women; the geometric

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**TABLE 1**

Descriptive characteristics of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>Lactating women</th>
<th>Nonlactating women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calcium (n = 38)</td>
<td>Placebo (n = 38)</td>
</tr>
<tr>
<td></td>
<td>Calcium (n = 40)</td>
<td>Placebo (n = 42)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>30 ± 3</td>
<td>31 ± 3</td>
</tr>
<tr>
<td>Parity</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Height (m)</td>
<td>164.5 ± 6.7</td>
<td>163.9 ± 6.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60.7 ± 14.0</td>
<td>61.4 ± 10.8</td>
</tr>
<tr>
<td>Dietary iron intake (mg/d)</td>
<td>13.2 ± 4.7</td>
<td>14.0 ± 4.2</td>
</tr>
<tr>
<td>Dietary vitamin C intake (mg/d)</td>
<td>69 (41, 114)</td>
<td>66 (34, 128)</td>
</tr>
<tr>
<td>Dietary calcium intake (mg/d)</td>
<td>684 ± 239</td>
<td>776 ± 222</td>
</tr>
<tr>
<td>Percentage of women who took ≥80% of pills (%)</td>
<td>92.1</td>
<td>94.7</td>
</tr>
<tr>
<td>Menses at enrollment</td>
<td>5/38</td>
<td>5/38</td>
</tr>
<tr>
<td>Percentage of women who completed weaning after enrollment (%)</td>
<td>92.1</td>
<td>94.7</td>
</tr>
<tr>
<td>Duration of iron supplement use postpartum, tertile distribution (%)</td>
<td>0–0.5 mo (n = 49)</td>
<td>13(^2)</td>
</tr>
<tr>
<td>0.5–4.0 mo (n = 56)</td>
<td>31.6(^2)</td>
<td>34.2(^2)</td>
</tr>
<tr>
<td>&gt; 4.0 mo (n = 53)</td>
<td>55.3(^2)</td>
<td>52.3(^2)</td>
</tr>
</tbody>
</table>

\(^1\) Women in the calcium and placebo groups received 500 mg Ca as calcium carbonate or placebo twice daily with meals, respectively. 
\(^2\) \( \bar{X} \pm SD \).
\(^3\) Significantly different from nonlactating women: \( P < 0.05 \), \( P < 0.01 \) (tested as the main effect of lactation).

\(^4\) Geometric mean (± 1 SD). Vitamin C intake does not include the 60 mg contained in the daily multivitamin supplement.

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Serum ferritin concentrations decreased significantly in lactating women compared with 2.3 μg/L compared with 7.1 ± 2.1 μg/L in nonlactating women (P < 0.01). By the end of the study (12 mo postpartum), there was no difference in serum ferritin concentrations between previously lactating women and nonlactating women; the geometric means were 30.5 and 25.5 μg/L, respectively (P = 0.14). Changes in hemoglobin concentration, MCV, and hematocrit over the study did not differ between lactating and nonlactating women whether or not previous postpartum iron supplementation or iron intake during the study was included in the analyses (P > 0.2).

We investigated whether the length of postpartum amenorrhea could account for some of the variability in serum ferritin concentration among lactating women and whether it was associated with the decreased serum ferritin concentrations after weaning. There was no difference in baseline serum ferritin concentrations among lactating women who had resumed menses before enrollment (n = 11) and those who had not (n = 65); the geometric means were 44.7 and 48.2 μg/L, respectively (P = 0.7). Furthermore, the length of postpartum amenorrhea in lactating women was not associated with the change in serum ferritin concentration during the 6-mo study (P > 0.3).

**DISCUSSION**

**Effects of calcium supplementation on iron status**

The goals of the present study were to determine whether long-term calcium supplementation has a deleterious effect on iron status in women in the postpartum period and to describe the effects of lactation and weaning on maternal iron status. We found that consumption of supplements containing 500 mg Ca twice daily for 6 mo did not alter serum ferritin concentrations, despite the fact that women were instructed to consume their calcium supplement at mealtime, a regimen that should have maximized the potential inhibitory effect of calcium on iron absorption. These findings provide reassurance that consumption of calcium supplements with the goal of optimizing bone health should not have the negative consequence of compromising iron status.

Several studies using radioisotopes to label iron in meals have shown that calcium inhibits both heme- and nonheme-iron absorption from a test meal and that the inhibitory effect occurs for a variety of calcium salts (3, 4, 6). Although calcium may decrease iron absorption from a test meal, our data indicate that daily calcium supplementation does not significantly reduce serum ferritin concentrations or result in overt iron deficiency. Our sample size was sufficient for detecting a minimum difference in serum ferritin concentration of ~7 μg/L between the calcium-supplemented and placebo groups with 80% power. Our findings are consistent with those from one other study in which calcium supplements were provided twice daily with meals, although the calcium dose used in that study was 250 mg Ca compared with 500 mg Ca in this study (11). In that study, both the iron intake (15.1 mg/d) and vitamin C intake (227 mg/d) of the study subjects were high, which may have overcome any potential inhibitory effect on iron absorption. Our failure to find an effect of calcium supplements on serum ferritin cannot be explained by an abundance of dietary iron compensating for reduced absorption efficiency because serum ferritin concentrations decreased among lactating women after weaning when menstrual blood loss resumed.

**FIGURE 1. Effects of calcium supplementation and lactation on serum ferritin concentrations.** Points represent the geometric mean ferritin concentration for each group. Women received 500 mg Ca as calcium carbonate or placebo twice daily with meals. Lactating women were breast-feeding an average of 5.4 times daily at baseline; the arrow labeled “weaned” indicates the average time of complete cessation of breast-feeding. Calcium effect (calcium group × time interaction), P = 0.8; lactation effect (lactation group × time interaction), P < 0.001. Serum ferritin concentrations were significantly greater for lactating women (mean difference = 0.8; lactation effect (lactation group × time interaction), P < 0.001). Serum ferritin concentrations decreased among lactating women after they weaned their infants, and the difference in serum ferritin concentrations between women who were previously lactating and nonlactating women diminished over time. The mean (±SD) decreases in serum ferritin concentrations at 9 and 12 mo postpartum (1.2 and 4.2 mo after complete weaning, respectively) were 13.9 ± 16.4 and 18.4 ± 17.5 μg/L for lactating women compared with 2.3 ± 17.7 and 6.2 ± 19.1 μg/L for nonlactating women. The percentage of lactating women with depleted iron stores (serum ferritin < 12 μg/L) increased from 6.7% at baseline when the women were fully lactating to 19.7% at the end of the study, an average of 4.2 mo after weaning (P = 0.03). The respective percentages for nonlactating women were 11.1% and 17.2% (P = 0.37). After adjustment for postpartum iron supplement use and dietary iron intake during the study, the mean (±SEM) decrease in serum ferritin concentration by 12 mo postpartum in women who were previously lactating was 17.6 ± 2.3 μg/L compared with 7.1 ± 2.1 μg/L in nonlactating women (P < 0.01).
There are several possible reasons we did not find an effect of long-term calcium supplementation on serum ferritin concentrations. The moderate amount of calcium in the diet may have been enough to maximally inhibit iron absorption so that no further inhibition occurred with calcium supplementation. The inhibitory effect of calcium on iron absorption from test meals has been shown to be dose dependent up to ~165–300 mg, with no further increase in inhibition between 300 and 600 mg Ca (5). It is also possible that women took their supplements between meals rather than with their meals as instructed, thereby minimizing the potential for inhibition of iron absorption.

Alternately, the test meal approach may overestimate the inhibitory effect of calcium on iron absorption, and the true inhibitory effect on iron absorption in the context of a complete diet may be small. Studies using isotopically labeled single test meals have found that calcium reduces iron absorption by ~50% (3, 4, 6), whereas the inhibitory effect is ~25% when iron absorption is measured from the whole diet (17, 18). Furthermore, balance studies in which subjects consume complete meals have generally not found an inhibitory effect of calcium on net iron absorption (19–21). The complex interplay of enhancing and inhibiting factors in a complete diet makes it difficult to predict the overall effect of diet on iron absorption.

Last, iron absorption appears to be a highly regulated process, although the exact mechanisms are unknown. Iron absorption is inversely related to iron stores and the serum ferritin concentration has been shown to be the most important determinant of iron absorption from a complete diet (22). It is possible that the initial inhibitory effect of calcium on iron absorption diminishes over time in the presence of daily calcium supplementation. Furthermore, it is not known whether low iron absorption at one meal enhances absorption at another.

One limitation of this study is that we measured indicators of iron status that best reflect the extremes in iron nutriture (ie, iron stores, microcytosis, and anemia) and did not measure indicators that reflect iron-deficient erythropoiesis, the intermediate stage of iron deficiency after depletion of iron stores (15). It is possible that we missed an effect of calcium supplementation on iron status that we could have detected by measuring serum transferrin receptor concentrations, transferrin saturation, or erythrocyte protoporphyrin. However, the subjects in this study were considered at risk for impaired iron stores, not iron-deficient erythropoiesis. We chose to measure serum ferritin concentrations because these are thought to best reflect iron stores and are the most sensitive indicator of iron status in well-nourished populations. Furthermore, we measured CRP to rule out potential spurious results due to increases in ferritin secondary to inflammation and infection (15, 16).

### Effects of lactation on iron status

We found that lactating women had greater serum ferritin concentrations, and presumably iron stores, than nonlactating women in the postpartum period and that serum ferritin concentrations decreased after weaning. The higher serum ferritin concentrations at baseline in lactating women may have been due in part to the increased use of iron supplements by lactating women before enrollment in the study. However, serum ferritin concentrations were still higher in lactating women after prior iron supplement use was statistically controlled for.

Prolonged postpartum amenorrhea in lactating women likely contributed to the differences in serum ferritin concentrations between lactating and nonlactating women. Only 14.5% of lactating women had resumed menses by 6 mo postpartum (baseline) whereas 98.8% of nonlactating women had resumed menses by this time. Menstrual blood iron loss is estimated to be 0.24 mg/d (23) in contrast with iron losses in breast milk of 0.24 mg/d (24). The drop in serum ferritin concentrations in lactating women after weaning may also have been due to the return of menses, which occurred in all but one of the participants by the final measurement taken in the study at 12 mo postpartum. We were unable to show a relation between the length of postpartum amenorrhea and change in serum ferritin concentration. It is possible that length of postpartum amenorrhea is too crude of a measure to adequately quantify the variability in iron loss with menses. Differences in volume of menstrual blood loss constitute the largest source of variability in iron status in menstruating women (23, 25). The variability in menstrual blood loss may be even greater postpartum because first menstrual cycles in lactating women may be anovulatory, irregular, and of short duration (26).

Whether other hormonal or metabolic changes that accompany lactation also contributed to the differences in serum ferritin concentration are unknown. Some acute phase proteins (eg, cerulo-
REFERENCES