

Iron, zinc, and copper concentrations in breast milk are independent of maternal mineral status¹⁻³

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ABSTRACT

Background: Little is known about the regulation of iron, zinc, and copper in breast milk and the transport of these minerals across the mammary gland epithelium.

Objective: The objective was to study associations between breast-milk concentrations of iron, zinc, and copper and maternal mineral status.

Design: Milk samples from 191 Swedish and Honduran mothers were collected at 9 mo postpartum. Iron, zinc, and copper concentrations were measured by atomic absorption spectrometry. Blood samples from mothers were analyzed for plasma zinc and copper and 4 indexes of iron status: hemoglobin, plasma ferritin, soluble transferrin receptors, and zinc protoporphyrin. Complementary food energy (CFE) intake was used as an inverse proxy for breast-milk intake.

Results: Mean (\pm SD) breast-milk concentrations of iron were lower in the Honduran than in the Swedish mothers (0.21 ± 0.25 compared with 0.29 ± 0.21 mg/L; $P < 0.001$), and mean breast-milk concentrations of zinc and copper were higher in the Honduran than in the Swedish mothers [0.70 ± 0.18 compared with 0.46 ± 0.26 mg/L ($P < 0.001$) and 0.16 ± 0.21 compared with 0.12 ± 0.22 mg/L ($P = 0.001$), respectively]. Milk iron was positively correlated with CFE intake ($r = 0.24$, $P = 0.001$) but was not significantly correlated with any iron-status variable. Milk zinc was negatively correlated with CFE intake ($r = -0.24$, $P = 0.001$) but was not significantly correlated with maternal plasma zinc. Milk copper was not significantly correlated with CFE intake or maternal plasma copper.

Conclusions: Milk iron, zinc, and copper concentrations at 9 mo postpartum are not associated with maternal mineral status, which suggests active transport mechanisms in the mammary gland for all 3 minerals. Milk iron concentrations decrease and milk zinc concentrations increase during weaning. *Am J Clin Nutr* 2004; 79:111-5.

KEY WORDS Human milk, breastfeeding, iron, zinc, copper, mammary gland

INTRODUCTION

The World Health Assembly recommends the exclusive breastfeeding of infants until 6 mo of age and continued breastfeeding with appropriate complementary feeding until 2 y of age (1). Iron deficiency and zinc deficiency are public health concerns during infancy, especially in developing countries (2, 3). Iron deficiency in infancy may lead to poor psychomotor

development (4, 5), and zinc deficiency may lead to stunted growth (6) and compromised immune function (7). Copper deficiency, as well as copper toxicity, is a concern in infancy, although precise copper requirements have not yet been established for this age group (8). Little is known about the mechanisms regulating the concentrations of iron, zinc, and copper in breast milk (9), although the proteins transporting these minerals across the mammary gland epithelium were recently characterized in cells and in animal studies. Iron is transported by divalent metal transporter 1 through the basolateral membrane into the alveoli and then is exported by ferroportin (also called IREG1) in the apical membrane (W Leong, B Lönnerdal, unpublished observations, 2001). Transferrin receptors are also likely to be involved in iron uptake (10, 11). Zinc uptake by the mammary gland probably occurs via ZTL1, ZIP1, or ZIP4, whereas the export into milk appears to be regulated by ZnT-2 and ZnT-4 (12). Copper uptake by the mammary gland is regulated by Ctrl, a membrane-associated transporter, whereas copper export is regulated by ATP7A (13, 14). The significance of these transporters in the regulation of trace element concentrations in human milk is not yet known.

We previously presented the results from a randomized controlled trial of >200 breastfed infants in Honduras and Sweden who were supplemented with iron; the primary endpoint was iron status at 9 mo (15). These infants were exclusively breastfed until 6 mo and at least partially breastfed to 9 mo. Blood samples were collected from the infants at 9 mo of age for the measurement of various indexes of iron status and for plasma zinc and copper concentrations.

Having access to breast-milk samples from a unique group of lactating mothers with a wide range of nutritional statuses allowed us to investigate whether there are any associations between milk concentrations of iron, zinc, and copper at 9 mo postpartum and maternal iron status or plasma zinc and copper concentrations. This was the aim of the current study. Modern

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indicators of iron status [plasma transferrin receptors (TfR) and zinc protoporphyrin (ZPP)] were included in the assessment of maternal iron status because the relations between these indicators and milk iron have not been investigated. Because socioeconomic conditions in Honduras and Sweden are very different, maternal iron status, zinc status, and possibly copper status were expected to differ between the 2 study sites.

SUBJECTS AND METHODS

Subjects

This study was conducted at 2 sites: San Pedro Sula (Honduras) and Umeå (Sweden) (15). Mother-infant pairs were recruited immediately after birth (in Honduras) or ≈ 3 mo postpartum (in Sweden). Inclusion criteria were as follows: 1) gestational age ≥ 37 wk, 2) birth weight >2500 g, 3) no chronic illness in the infant, 4) maternal age ≥ 16 y, and 5) infant exclusively breastfed at 4 mo. Between 4 and 6 mo, the mothers were discouraged from giving their infants any other foods or fluids, except for "taste portions" [≤ 1 Tbsp (15 mL)/d] of foods with little or no iron. Between 6 and 9 mo, the mothers continued breastfeeding but were allowed to give complementary food at their own discretion. No attempt was made by the investigators to influence the choice of foods or the extent of breastfeeding. The study was approved by the Human Subjects Review Committee of the University of California, Davis, and the Ethical Committee, Faculty of Medicine and Odontology, Umeå University, Sweden. All participating mothers gave written informed consent.

Data collection and analysis

Breast-milk samples (10–40 mL) were collected at 9 mo postpartum. Samples were collected in the morning ≥ 1 h after the previous breastfeeding. Breast milk was expressed by hand or manual pump from one breast into plastic containers provided by the investigators. Single-use plastic containers were tested and found negative for mineral contamination. Plastic pumps were washed in 0.1% nitric acid before being reused and were tested and found negative for mineral contamination after being washed. Samples were transferred to zinc-free, screw-cap, plastic tubes; frozen at -20 °C; and transported to the University of California, Davis, for analysis. Milk samples were wet ashed and analyzed according to Clegg et al (16) with some minor modifications. Samples were thawed, mixed thoroughly, and digested in ultrapure concentrated nitric acid (Fisher, Los Angeles) at room temperature for 96 h and then at subboiling temperature for 6–9 h. Iron, zinc, and copper concentrations were determined by atomic absorption spectrometry (model Smith-Heijfjie 4000; Thermo Jarrell Ash Corporation, Franklin, MA). Elemental standards from Fisher Scientific International Inc (Los Angeles) and standard reference materials from the National Institute of Standards and Technology (Gaithersburg, MD) were used. The analyzed reference values were within 95–105% of the certified value.

Venous blood (≈ 5 mL) was obtained from the mothers at 9 mo postpartum. In Honduras, maternal blood samples were generally obtained in the morning after an overnight fast. In Sweden, the samples were generally taken in the morning, but mothers were not necessarily fasting. Part of the sample was collected in an EDTA-treated test tube and immediately ana-

lyzed in duplicate for hemoglobin (HemoCue, Ängelholm, Sweden) and ZPP (Protofluor Z; Helena Labs, Beaumont, TX). These 2 instruments were checked weekly against standard solutions at both sites. The other part of the sample was collected in a lithium heparin-treated tube and, after centrifugation ($1750 \times g$, 10 min, room temperature), plasma was stored frozen at -20 °C until analyzed for ferritin (Coat-A-Count Ferritin IRMA; Diagnostic Products Corp, Los Angeles) and TfR (Ramco, Houston). Plasma samples were diluted 1:5 (by vol) in 1% nitric acid, the samples were allowed to undergo digestion for 2 d, and concentrations of zinc and copper were determined by atomic absorption spectrometry as described above.

Complementary food intakes between 6 and 9 mo were estimated in Honduras by a biweekly 24-h dietary recall and a food-frequency questionnaire and in Sweden by a monthly 5-d food diary. Nutrient intakes in Honduras were calculated by using the FOOD PROCESSOR program (ESHA Research, Salem, OR), and those in Sweden were calculated by using food-composition tables from the National Food Administration and information from Swedish baby food manufacturers. Infant weight at 9 mo of age was measured by the investigators.

Statistics

All statistical analyses were performed by using SPSS software version 11.0 (SPSS Inc, Chicago). Student's *t* test was used for comparing means, Fisher's exact test for comparing proportions, linear regression analysis for studying correlations, and analysis of covariance for studying correlations while potential confounders were controlled for.

Because the distributions of plasma ferritin, ZPP, milk iron, milk zinc, milk copper, and complementary food energy intake were skewed, these variables were log transformed in all calculations. For presentation, the variables were transformed back to the original scale and presented as geometric means. To assess the possible predictors of milk mineral concentrations, multiple regression analyses were performed, including the following explanatory variables: 1) maternal mineral status (plasma zinc and copper or an index of iron status), 2) complementary food energy intake (inverse proxy for breast-milk intake), and 3) study site (Honduras or Sweden).

RESULTS

Of the 263 mother-infant pairs recruited at 4 mo postpartum, 214 remained in the study at 9 mo postpartum. The total dropout rate was not significantly different by study site. Considering both study sites separately, there were no significant differences between dropouts and nondropouts with respect to maternal age, weight, height, parity, infant sex, or birth weight. The most common reasons for dropout were suspected side effects of the iron or placebo drops ($n = 12$) and the family moving out of the study area ($n = 8$). Of the mothers remaining in the study at 9 mo, 21 could not produce a sufficient breast-milk sample (10 mL) and 2 refused blood sampling. Sufficient breast-milk and blood samples were obtained from 191 mothers, and data from these subjects were included in the statistical analysis.

Compared with the Swedish mothers, the Honduran mothers were significantly younger, were of higher parity, and had lower weights and heights, and their infants had lower weights

TABLE 1
Background characteristics of the subjects by site¹

| | Honduras (<i>n</i> = 105) | Sweden (<i>n</i> = 86) |
|----------------------|-------------------------------|----------------------------|
| Maternal age (y) | 26 ± 6 | 31 ± 4 ² |
| Parity (<i>n</i>) | 2.8 ± 1.9 | 1.8 ± 0.9 ² |
| Maternal weight (kg) | 56 ± 11 | 65 ± 10 ² |
| Maternal height (cm) | 151 ± 6 | 166 ± 6 ² |
| Birth weight (kg) | 3.2 ± 0.4 | 3.6 ± 0.4 ² |
| Birth length (cm) | 49 ± 2 | 51 ± 2 ² |

¹ $\bar{x} \pm \text{SD}$.² Significantly different from Honduras, $P < 0.001$ (two-tailed *t* test, independent samples).

and lengths at birth (Table 1). The mean (\pm SD) complementary food energy intake of infants was lower in Honduras than in Sweden (25 ± 11 compared with 41 ± 22 kcal · kg⁻¹ · d⁻¹; $P < 0.001$). There was a significantly higher proportion of Honduran than of Swedish mothers with low hemoglobin (12% compared with 0%), low ferritin (32% compared with 12%), and high ZPP (6% compared with 0%), whereas the proportions of mothers with high TfR concentrations were not significantly different between sites (5% compared with 4%) (Table 2). Infant birth weight, maternal age, parity, weight, and height were not significantly correlated with any of the main outcome variables when site was adjusted for. Therefore, these variables were not included as potential confounders in subsequent analyses.

The Honduran mothers had significantly lower breast-milk iron concentrations than did the Swedish mothers (Table 3). After control for study site and complementary food energy intake, no significant correlations between milk iron concentration and any of the indexes of maternal iron status were observed: hemoglobin ($r = -0.03$, $P = 0.73$), ferritin ($r = -0.06$, $P = 0.41$), ZPP ($r = 0.00$, $P = 0.99$), and TfR ($r = -0.05$, $P = 0.44$). Furthermore, there was no significant difference in the mean milk iron concentration between anemic and nonanemic Honduran mothers (0.26 compared with 0.21 mg/L; $P = 0.23$). However, there was a positive correlation between milk iron concentration and complementary food energy intake ($r = 0.24$, $P = 0.001$) after control for the study site, which suggests that milk iron concentrations increase with weaning. When the 2 study sites were analyzed separately, no significant correlations in Sweden or Honduras between milk iron concentration and any of the iron- status variables were observed after control for complementary food energy intake. In Honduras, there was a positive correlation between milk iron

TABLE 2
Proportion of mothers fulfilling criteria for iron deficiency by site

| Deficiency cutoff | Honduras (<i>n</i> = 105) | Sweden (<i>n</i> = 86) |
|--|-------------------------------|----------------------------|
| | % | |
| Hemoglobin < 120 g/L | 12 | 0 ¹ |
| Ferritin < 12 μg/L | 32 | 12 ¹ |
| Zinc protoporphyrin > 80 μmol/mol heme | 6 | 0 ² |
| Transferrin receptor > 8.3 mg/L | 5 | 4 |

^{1,2} Significantly different from Honduras (Fisher's exact test, two-tailed): ¹ $P = 0.001$, ² $P = 0.033$.**TABLE 3**
Iron, zinc, and copper concentrations in the breast milk of mothers by site¹

| | Honduras (<i>n</i> = 105) | Sweden (<i>n</i> = 86) |
|--------|-------------------------------|----------------------------|
| | mg/L | |
| Iron | 0.21 ± 0.25 | 0.29 ± 0.21 ² |
| Zinc | 0.70 ± 0.18 | 0.46 ± 0.26 ² |
| Copper | 0.16 ± 0.21 | 0.12 ± 0.22 ³ |

¹ Geometric $\bar{x} \pm \text{SD}$.^{2,3} Significantly different from Honduras (two-tailed *t* test, independent samples): ² $P < 0.001$, ³ $P = 0.001$.

concentration and complementary food energy intake ($r = 0.30$, $P = 0.002$). This correlation was not significant in the Swedish subsample ($r = 0.17$, $P = 0.116$).

The Honduran mothers had significantly higher breast-milk zinc concentrations than did the Swedish mothers (Table 3). After control for study site and complementary food energy intake, there was no significant correlation between milk zinc concentration and maternal plasma zinc ($r = -0.06$, $P = 0.61$). After control for study site, milk zinc concentration was negatively correlated with complementary food energy intake ($r = -0.24$, $P = 0.001$), which suggests that milk zinc concentrations decrease with weaning. When the 2 study sites were analyzed separately, no significant correlation in Sweden or Honduras between milk zinc concentration and maternal plasma zinc was observed after control for complementary food energy intake. In Sweden, there was a negative correlation between milk zinc concentration and complementary food energy intake ($r = -0.27$, $P = 0.012$). This correlation was not significant in the Honduran subsample ($r = -0.19$, $P = 0.058$).

The Honduran mothers had significantly higher breast-milk copper concentrations than did the Swedish mothers (Table 3). In the multivariate analysis that included maternal plasma copper, complementary food energy intake, and study site as explanatory variables, there was no significant correlation between milk copper concentration and maternal plasma copper ($r = 0.12$, $P = 0.08$) or between milk copper concentration and complementary food energy intake ($r = 0.14$, $P = 0.069$). When the 2 study sites were analyzed separately, there was no significant correlation in Sweden or Honduras between milk copper concentration and maternal plasma copper, after control for complementary food energy intake. In Honduras, a positive correlation between milk copper concentration and complementary food energy intake ($r = 0.24$, $P = 0.012$) was observed. This correlation was not observed in the Swedish subsample ($r = 0.03$, $P = 0.76$).

DISCUSSION

Previous studies have shown no correlation between maternal iron status and milk iron concentration (17, 18). Furthermore, maternal dietary iron appears to have little effect on human milk iron concentration (19). A single study has shown increased concentrations of iron in the milk from severely anemic mothers (20). Unlike previous studies, the current study included a larger number of lactating mothers, a wider range of maternal nutritional status, and an analysis of TfR, which is a


novel indicator of iron status. As found in most previous studies, we found no correlation between milk iron and any of the iron-status variables (hemoglobin, ferritin, ZPP, and TfR). Our results suggest that the site difference in milk iron concentrations (lower in Honduras) was caused by differences in milk volume (higher in Honduras) rather than by differences in maternal iron status.

Our observation that milk zinc concentrations were >50% higher in Honduras than in Sweden was unexpected because the Honduran mothers had significantly lower plasma zinc concentrations. However, the multivariate analysis showed that the site difference in milk zinc concentration was attributable to differences in milk volume rather than to differences in maternal plasma zinc. This finding is consistent with the observation by Moser et al (21) of similar milk zinc concentrations in Nepali and American women despite lower plasma zinc concentrations in the Nepali women. It is well known that plasma zinc concentrations decrease after a meal (22). In Honduras, maternal blood samples were obtained in the morning after an overnight fast. In Sweden, the samples were generally taken in the morning, but mothers were not necessarily fasting. This difference in study procedure is not likely to explain the differences in plasma zinc between study sites, because it would have resulted in higher rather than lower plasma zinc concentrations in Honduras.

It is interesting that prolonged breastfeeding and low complementary food intake in Honduras were associated with higher zinc concentrations in breast milk from Honduran women, despite lower maternal plasma zinc concentrations. This may be an important factor in the prevention of zinc deficiency among breastfed infants in developing countries. Most previous studies found no correlation between maternal dietary zinc intake and milk zinc concentration (19, 23). A single study showed a slight increase in milk zinc after zinc supplementation (24), but a subsequent, larger study by the same researchers showed no effect on milk zinc (25). However, it is likely that most of the women in those studies had adequate zinc status. There are few human studies on milk zinc in women with low zinc status, although this has been explored in animal studies. When pregnant and lactating rats were fed a diet marginal in zinc, no effect on milk zinc was found (26). In lactating rats with marginal zinc deficiency, we found significant up-regulation of the expression of ZnT-2 and ZnT-4, which would be a compensatory mechanism to maintain milk zinc concentrations (12).

Previous studies found no effect of dietary copper on the copper concentration of human milk (19). In our study, we found no correlation between maternal plasma copper and milk copper. It is interesting that milk copper was higher in the Honduran than in the Swedish women. We found that milk copper was significantly elevated in zinc-deficient lactating rats and that this increase was associated with increased expression of Ctr1 and ATP7A (12). The mechanism behind this up-regulation of milk copper transport during zinc deficiency is not yet known. The observed site difference in milk copper (higher in Honduras) cannot be explained by the observed positive correlation between complementary food energy intake (lower in Honduras) and milk copper concentration in the Honduran subsample. We conclude that milk copper concentrations are determined by factors other than maternal plasma copper and milk volume.

If the passage of iron, zinc, and copper from plasma to milk in the mammary gland were the result of passive diffusion, positive correlations would be expected between plasma mineral status (concentration) and milk mineral concentration. Because we found no such correlations, our results suggest a regulated transport of iron, zinc, and copper through the mammary gland epithelium. The findings of recent animal studies agree with such an interpretation (12).

The concentrations of some nutrients in breast milk (eg, fat, protein, and sodium) increase during weaning, whereas others (eg, lactose and calcium) decrease (27). We found that a high complementary food energy intake (weaning) was associated with low milk zinc and high milk iron concentrations, which suggests that these 2 minerals are affected differently by weaning. This finding is supported by a previous study by our group in which breast-milk concentrations of zinc decreased and those of iron increased in late lactation (27). Decreased milk zinc and increased milk iron with increasing complementary food energy intake result in a decreased ratio of the concentrations of zinc and iron in breast milk during the process of weaning. The consequences of this change in ratio, if any, are unknown. We speculate that the increase in milk iron during weaning is explained by the fact that milk protein concentrations increase during weaning, and a large proportion of iron is bound to one of the milk proteins, namely lactoferrin (28). Milk zinc, however, is found largely in the low-molecular-weight fraction (associated with citrate), the concentration of which is associated with lactose and water transport, both of which decrease during weaning (28). Further studies of mammary gland physiology during weaning are needed to explain these observations. 

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RJC collected the Honduran data, and MD collected the Swedish data. BL was responsible for all of the laboratory analyses. MD analyzed the data. MD, BL, KGD, and OH were responsible for the study design and the preparation of the manuscript. None of the authors had a conflict of interest to report.

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