Changes in Zinc and Copper Concentrations in Breast Milk and Blood of Japanese Women during Lactation

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Summary To evaluate the changes in zinc and copper concentrations in breast milk and maternal blood during lactation, milk and blood samples were obtained from 80 lactating women during the period between 2 and 201 days of lactation. Zinc and copper concentrations were measured by atomic absorption spectrophotometry. Breast milk zinc and copper concentrations markedly decreased during the first few weeks of lactation and gradually declined for the remaining period. Mean values of milk zinc and copper levels were 1.76 and 0.29μg/ml, respectively, between 15 and 84 days after parturition and were 0.76 and 0.19μg/ml between 85 and 201 days of lactation. Calculated daily intakes of these minerals for infants from breast milk were markedly lower than those of US Recommended Dietary Allowances. Plasma zinc levels of lactating mothers increased as lactation progressed, whereas erythrocyte zinc and plasma copper concentrations decreased. Plasma zinc and copper and erythrocyte zinc values returned to normal approximately three months after parturition.

Key Words zinc, copper, human milk, plasma, erythrocyte, lactation, intake, requirement, infants, lactating women

Zinc and copper are essential trace minerals serving a wide range of functions including the structural integrity of cells and a part of many enzymes and other molecules (1). Zinc plays an important role in the metabolism of protein, nucleic acids and carbohydrates; therefore, it is important for normal growth and development (2). Copper also plays an essential role in a wide range of cellular activities and is important for growth and reproduction (3).

Since the first description of human zinc deficiency in 1963 (4), many reports have appeared describing marginal to severe zinc deficiency in infants and children (5–19). Among these, several reports indicated that overt zinc deficiency occurs in
early infancy probably due to an abnormally low zinc concentration in breast milk (8–10, 13, 15, 17, 18). Also, in the last two decades, many investigators reported cases of copper deficiency during infancy and childhood (20–29). Therefore, the importance of these minerals in infant nutrition is well recognized.

There are a number of reports concerning zinc and copper concentrations in human milk from various countries (30–50) and extensive reviews have been published (51, 52). We have investigated the changes in breast milk zinc and copper concentrations in healthy, well-nourished Japanese lactating women. In this report, we discuss the estimated intakes of these minerals in their infants. In addition, we describe the changes in maternal blood concentrations of zinc and copper during the course of lactation.

MATERIALS AND METHODS

Subjects. A total of 80 lactating women (age range: 19–37 years) participated in the study. They were living in a city located in the northern part of mainland Japan with similar socioeconomic backgrounds and were apparently healthy at the time of the study with no history of serious diseases. They were asked to maintain their regular diet before and during the study, although dietary histories were not collected. Informed consent was obtained from each subject after the purpose and procedures of the project were explained. This study was carried out in general accordance with the Helsinki Declaration.

The samples were obtained during the regular monthly checkup for infants at the pediatric clinic, and at the hospital where deliveries were carried out for mothers whose infants were less than 11 days old. Some of the subjects participated two to three times for the collection of the samples. All infants were healthy and exhibited normal growth and development, and their age ranged between 2 and 201 days. Not all infants were totally breast-fed. Measurement of heights and weights of infants was carried out simultaneously with sample collection.

Samples. Approximately 5 ml of breast milk was collected by manual expression directly into acid-washed glass tubes in early afternoon (2:00–3:00 PM) immediately before the infants were fed. Milk samples were kept at room temperature and were analyzed within 4 days after collection. Storage of the samples at 4°C or −20°C was not suitable for assay, as the rate of sample aspiration to the instrument decreased significantly.

Breast milk zinc and copper levels were analyzed in 6 subjects at the beginning and the end of feeding. The zinc values did not differ significantly during the emptying of the breasts (at the beginning, 5.61±2.46 μg/ml; at the end, 5.90±2.25 μg/ml; M±SD) and similar findings were observed in copper concentrations (at the beginning, 0.40±0.10 μg/ml; at the end, 0.42±0.12 μg/ml). Therefore, we concluded that zinc and copper concentrations of milk collected at the beginning of breast feeding represent those throughout feeding.

Maternal blood samples were obtained using heparinized plastic syringes
immediately before the collection of milk samples. After the measurement of hematocrit, aliquots of whole blood were mixed with 9 volumes of distilled-deionized water for erythrocyte zinc determinations. The calculation of erythrocyte zinc concentrations was made using the formula: \[ \text{whole blood concentration} - \text{plasma concentration} \times (1 - \text{hematocrit} / 100) \] \div (\text{hematocrit} / 100). Plasma samples and hemolysates were stored at \(-20^\circ\text{C}\) until assayed.

Determinations of zinc and copper. Zinc and copper concentrations in milk, plasma and hemolysate samples were measured using atomic absorption spectrophotometry (Model 1100, Varian-Techtron Pty. Ltd., Melbourne, Australia). The details of the procedures were previously described (53). The recoveries of known amounts of zinc or copper added to milk samples ranged between 97 and 104\% for zinc and 96 and 104\% for copper.

RESULTS

As shown in Fig. 1, breast milk zinc concentrations decreased as the duration

![Fig. 1. Changes in breast milk zinc concentrations during lactation. The best fitting curve is represented by \( [\text{milk zinc}] = 14.9 \times [\text{days of lactation}]^{-0.60} \) with a correlation coefficient of \(-0.89 \) \((p<0.0001)\).]
of lactation increased. The greatest decline was within 14 days after parturition. Zinc levels gradually decreased thereafter until around 84 days of lactation when the decline subsided. Copper concentrations in breast milk also decreased as the stage of lactation progressed, while the decline was not as great as that of zinc as shown in Fig. 2. Copper concentrations gradually decreased until about the 84th day of lactation and became constant. It should be noted that the individual variations in the concentrations of milk zinc and copper was large particularly in colostrum. A similar pattern of the decreases in both zinc and copper concentrations in breast milk was observed, when the same subjects were examined two to three times at monthly intervals (Fig. 3A and B). Both zinc and copper concentrations in breast milk at various stages of lactation are summarized in Table 1.

Immediately after the delivery, maternal plasma zinc concentrations were significantly lower than the literature values of nonpregnant females in childbearing age (2). These increased rapidly for approximately 40 days after parturition (Fig. 4A). On the contrary, erythrocyte zinc levels gradually declined throughout the period studied (Fig. 4B). Copper concentrations in maternal plasma were markedly

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**Fig. 2.** Changes in breast milk copper concentrations during lactation. The best fitting line is \([\text{milk copper}] = -0.0012 \times [\text{days of lactation}] + 0.36\) with a correlation coefficient of \(-0.54\) \((p < 0.0001)\).
higher when compared to nonpregnant women at childbearing age who are not taking oral contraceptives (3), and declined rapidly within 2 weeks after delivery. The values continued to decrease until around 3 months of lactation, and maintained a constant level thereafter (Fig. 5).

**DISCUSSION**

Breast milk zinc and copper concentrations varied considerably among individuals and decreased as the stage of lactation progressed. This observation is consistent with previous findings by other investigators who uniformly described the declines in concentrations of these minerals during lactation in many parts of
Changes in plasma (A) and erythrocyte (B) zinc concentrations during lactation. The best fitting curve for changes in plasma zinc is represented by 
\[ \text{plasma zinc} = \text{days of lactation} \times (2.44 + 1.11 \times \text{days of lactation}) \] with a correlation coefficient of 0.77 (p<0.0001), and in erythrocyte zinc by 
\[ \text{erythrocyte zinc} = -0.023 \times \text{days of lactation} + 19.5 \] with a correlation coefficient of -0.58 (p<0.0001).

Concentrations of zinc and copper in colostrum were high and rapidly decreased in the first 2 to 3 weeks after parturition and then stabilized in the later days of lactation. It is unknown why the levels of both trace minerals are high in colostrum. There are two possible explanations including: 1) that newborn infants require larger amounts of these minerals relative to the volume of milk intake in their early life as compared to later days; and 2) that the milk-binding protein of these metals changes during the course of lactation. The latter possibility is supported by the findings of Suzuki et al. (49) indicating that the distribution of zinc-bound proteins in human milk drastically changes in the early stage of lactation.

It is generally agreed that breast milk is the best food for infants in their early life, and it is believed that the assessment of nutrient requirements in infants can be based on the composition and volume of breast milk from healthy, well-nourished mothers having healthy breast-fed infants with normal growth (54). We estimated the daily zinc intake through breast milk for infants by calculating the zinc content, amount of milk consumed by infants based on energy intakes (breast milk, 75 kcal/100 ml; energy intake, 100–120 kcal/kg of body weight per day), and average body
Fig. 5. Changes in plasma copper concentrations during lactation. The best fitting curve is $\text{[plasma copper]} = -0.32 \times \log_e \text{[days of lactation]} + 2.48$ with a correlation coefficient of $-0.87 \ (p < 0.0001)$.

Table 2. Ranges of estimated zinc and copper intakes from breast milk concentrations, and body weight and height of infants.

<table>
<thead>
<tr>
<th>Days of lactation</th>
<th>Zinc intake (µg/kg/day) (mg/day)</th>
<th>Copper intake (µg/kg/day) (mg/day)</th>
<th>Body weight (kg)</th>
<th>Height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8–14</td>
<td>525–630 1.7–2.1</td>
<td>52–62 0.17–0.21</td>
<td>3.3±0.6</td>
<td>51.8±1.3 (8)</td>
</tr>
<tr>
<td>15–84</td>
<td>235–282 1.2–1.4</td>
<td>39–46 0.28–0.24</td>
<td>5.1±0.9</td>
<td>56.3±3.2 (28)</td>
</tr>
<tr>
<td>85–201</td>
<td>101–122 0.75–0.90</td>
<td>25–30 0.19–0.22</td>
<td>7.4±1.2</td>
<td>63.6±3.5 (25)</td>
</tr>
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* M±SD (number of cases determined).

weight of infants (55). As summarized in Table 2, daily zinc intakes ranged between 525 and 630 µg/kg of body weight during the second week of life and decreased gradually to 235 to 282 µg/kg until 3 months of age. It was an unexpected finding that the estimated daily intakes of zinc further declined to between 101 and 122 µg/kg for infants older than 84 days. These calculated values of zinc intake are
not sufficient to meet the US Recommended Dietary Allowances (RDA), which suggests that intake should be 5 mg per day for less than 12 month-old infants (56). It is known that breast milk zinc is highly available for infants as compared to the availability of cow's milk zinc (57). Even if we conservatively assume that zinc in formula milk or infant food is only 20% available to infants and zinc in human milk is 100% available, the US RDA seems to be unrealistically high compared to the zinc intakes of Japanese infants in this study. At around 3–6 months of age, infants are generally introduced to beikost which may contain substantially larger amount of zinc.

Based on the recent report by Walravents et al. (19) indicating that zinc supplementation increased the growth of breast-fed male infants in both weight and height, zinc intakes of totally breast-fed infants may not be sufficient to meet their requirements for normal growth and development. Since their findings suggest the presence of marginal zinc deficiency among apparently healthy breast-fed infants, it is important to pay more attention to the relationship between the concentrations of breast milk zinc and the growth of breast-fed infants. As pointed out by Matsuda and Higashi (58), however, the infants nursed by formulas may have sufficient intakes, since the concentrations of zinc as well as copper in formulas are sufficient to supply the requirements of these minerals for infants in certain countries.

Daily intakes of copper through breast milk obtained in our study ranged between 52 and 62 μg/kg for infants during the second week of life and between 39 and 46 μg/kg for 15 to 84 day-old infants. These values were also markedly lower than the Estimated Safe and Adequate Daily Dietary Intakes proposed by the National Research Council (56), where 0.4–0.6 mg/day of copper intake was recommended for infants less than 6 months old and 0.6–0.7 mg/day for infants between 6 and 12 months old. As stated for zinc requirements for infants, it may be necessary to carefully examine adequacy of copper intakes for totally breast-fed infants.

Although a number of investigators have reported changes in maternal blood zinc and copper concentrations during pregnancy, to our knowledge, these reports on lactation are scarce, particularly when the concentrations of the minerals change drastically during the first few weeks of lactation (38, 41, 42, 59, 60). It is well known that plasma zinc levels steadily decline throughout pregnancy; however, the exact mechanism of this phenomenon is not known (2). It is also unclear why the changes occur in zinc concentrations in maternal plasma and erythrocytes during the early stages of lactation as observed in this study. Our findings are consistent with the previous observations indicating that the high levels of plasma copper, caused by increased estrogen levels during pregnancy, return to normal within a few weeks after parturition (3). It is important to consider these changes when evaluating the nutriture of lactating women based on blood zinc or copper levels. The concentrations of maternal plasma zinc and copper, and erythrocyte zinc presented here may be used as a reference for future studies.
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REFERENCES


