Zinc and Copper Contents in Breast Milk of Japanese Women

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HIGASHI, A., IKEDA, T., UEHARA, I. and MATSUDA, I. Zinc and Copper Contents in Breast Milk of Japanese Women. Tohoku J. exp. Med., 1982, 137 (1), 41-47 — Longitudinal studies of zinc and copper contents of breast milk were performed in 65 Japanese mothers during their lactation period until 5 months postpartum. They experienced no problems during pregnancy and at delivery. All women had full term healthy infants. Forty-five were multiparous and 20 primiparous. The highest level of zinc content was found in the colostrum, and subsequently the levels declined \( p<0.005 \), as lactation progressed. Copper content was stable during the first month of lactation, and then declined gradually \( p<0.005 \). Both mineral levels in breast milk ranged with great variance among the subjects at any stages of lactation. Significantly lower zinc level \( p<0.01 \) and higher copper level \( p<0.005 \) were found in the serum of lactating women three months postpartum, when compared with non-lactating control women. Factors including lactation history, age of lactating women, serum levels and contents of zinc and copper in the hair did not affect the contents of these trace minerals in the breast milk.

Recently, the importance of trace elements including zinc and copper have been established in human nutrition and medicine in the field of pediatrics (Committee on Nutrition 1978; Shaw 1979, 1980). The World Health Organization Expert Committee on Trace Element in Human Nutrition (1973), Committee on Nutrition of American Academy of Pediatrics (1976), and European Society for Pediatrics Gastroenterology and Nutrition (1977) have recommended that certain amounts of trace minerals are needed for infant formula feeding. In Japan, however, zinc and copper contents of infant formulas are less compared to those recommendations (Matsuda et al. 1979), and no addition of the trace minerals to formulas is permitted by the Ministry of Health and Welfare of Japan. We designed the present survey based on two reasons: the first, longitudinal study of trace elements in breast milk, especially of Japanese women, is lacking, and this sort of study is necessary before evaluating the above recommendation in Japan, and the second we intend to seek factors effectible for trace element level in breast milk, since a

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few studies (Piccano and Guthrie 1976; Vaughan et al. 1979) have been performed regarding this aspect.

**SUBJECTS AND METHODS**

Sixty-five Japanese women from 21 to 37 years of age (mean 27.3) were the subjects of the present study. All subjects had delivered healthy full term infants more than 2,500 g and did not experience any complication during pregnancy and at delivery. Twenty subjects were primipara and 45 were multipara. The nutritional condition of each subject was judged to be good, based on clinical observations. Breast milk samples were obtained longitudinally at the first lactation (colostrum), one week (transitional milk), one month, 3 months and 5 months postpartum (mature milk).

These were collected at morning by manual milking before the baby was due to be fed. Approximately 10 ml were obtained directly into clean polyethylene bottles, after breasts were cleaned twice with deionized water to avoid variations in components of milk from contamination (Vuori and Kuitunen 1979). Hair samples and serum specimens were collected one week and 3 months postpartum, respectively, for their trace mineral analyses.

Nineteen age matched healthy women served as control subjects for detecting serum concentrations of zinc and copper.

Milk and serum samples were diluted with deionized water for determination of zinc and copper by atomic absorption spectrophotometry (Parkin-Elmer Model 403) (Ohtake 1977; Rajalakshmi and Srikantia 1980). Pilot study indicated that when known amounts of minerals were added to milk or serum, 96% or more of added minerals were recovered.

Approximately 100 mg of hair was cut by stainless steel scissors from the nape of neck, as close to the scalp as possible. Hair samples were prepared by washing first in a sulfonated fatty acid detergent, followed by a rinse with deionized water and two ethanol solutions. After a final rinse in ethyl ether, hair samples were dried and ashed by the low temperature decomposition using excited oxygen (Gleit and Holland 1962). Ashed samples were diluted with 0.1 N-nitric acid for determination of zinc and copper by atomic absorption spectrophotometry.

Statistical comparisons of mean levels of data were made using Student’s t test. Linear regression analysis was performed on distributed data.

**RESULTS**

**Zinc and copper contents in breast milk**

Zinc and copper contents in 275 milk samples on different stages of postpartum are shown in Table 1. They showed a large variance of individual samples at any date of studies. The highest zinc level was found in the colostrum. Subsequently zinc content declined gradually and significantly \((p<0.005)\) during the first 3 months of lactation and then came to a plateau level. On the other hand, copper contents remained unchanged during the first month of lactation. No significant differences were noted between the copper levels in colostrum and in milk samples obtained one week and one month postpartum. Subsequently, however, copper levels declined significantly \((p<0.005)\) as lactation progressed.

The effect of the age of lactating women on zinc and copper contents of milk samples was studied. Significant negative correlation was found between the age and zinc content in breast milk obtained 3 months postpartum (Fig. 1), but not at any other occasions. Lactation history, primipara and multipara states, did not influence the zinc or copper content at any stage of lactation (Table 2).
Zinc and copper contents in maternal serum and hair

Zinc and copper contents in 44 maternal sera were measured at 3 months postpartum (Table 3). Serum zinc levels of lactating women ranged from 0.55 µg/ml to 1.15 µg/ml and these were significantly lower than those of non-lactating control women (p<0.01). On the other hand, serum copper levels of lactating

<table>
<thead>
<tr>
<th>Number of samples</th>
<th>Zinc (mg/liter)</th>
<th>Copper (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostrum 65</td>
<td>10.39±4.43µg/ml</td>
<td>0.45±0.23µg/ml</td>
</tr>
<tr>
<td>1 week 65</td>
<td>4.56±3.01µg/ml</td>
<td>0.45±0.15µg/ml</td>
</tr>
<tr>
<td>1 month 65</td>
<td>2.66±1.03µg/ml</td>
<td>0.44±0.10µg/ml</td>
</tr>
<tr>
<td>3 months 45</td>
<td>1.14±0.67µg/ml</td>
<td>0.29±0.09µg/ml</td>
</tr>
<tr>
<td>5 months 35</td>
<td>1.05±0.46µg/ml</td>
<td>0.22±0.08µg/ml</td>
</tr>
</tbody>
</table>

Mean±s.d.
* Not significant. † Significantly different (p<0.005).

Fig. 1. Relationship between the age of lactating women and zinc content in breast milk.

Zinc and copper contents in maternal serum and hair

Zinc and copper contents in 44 maternal sera were measured at 3 months postpartum (Table 3). Serum zinc levels of lactating women ranged from 0.55 µg/ml to 1.15 µg/ml and these were significantly lower than those of non-lactating control women (p<0.01). On the other hand, serum copper levels of lactating
women were significantly higher than those of non-lactating women (Table 3). No positive correlation of either in zinc content or in copper content was found between serum and milk samples at 3 months of lactation. Zinc and copper levels in hair samples ranged from 56.0 to 480.0 µg/g (mean 206.2 µg/g) and from 2.8 to 23.5 (mean 8.8 µg/g) respectively. Three of the 64 cases had hair levels below 100 µg/g of zinc content and they showed 1.0, 1.3 and 1.6 mg/liter of zinc contents in their milk samples at one month postpartum, corresponding to the lowest levels in the present study. But, when all samples were taken together, no significant correlation in zinc and copper contents between hair and milk samples was obtained at any stage of lactation.

**DISCUSSION**

Earlier studies were done mostly with cross-sectional or pooled samples (Belavady 1978; Murthy and Rhea 1971; Piccano and Guthrie 1976; Rajalakshmi and Srikantia 1980). Only a few studies, including the present one, were performed longitudinally (Vaughan et al. 1979; Vuori and Kuitunen 1979). They showed considerable variation in both zinc and copper contents of breast milk from subject to subject and at different stages of lactation. The highest levels of both trace minerals were in colostrum, and the levels declined gradually as lactation progressed (Belavady 1978; Murthy and Rhea 1971; Piccano and Guthrie 1976; Rajalakshmi and Srikantia 1980; Vaughan et al. 1979; Vuori and Kuitunen 1979). Both levels at different lactating stages in the present study were compatible with those of earlier international data (Belavady 1978; Rajalakshmi and Srikantia 1980; Vaughan et al. 1979; Vuori and Kuitunen 1979). Picciano

**TABLE 2. Zinc and copper contents in breast milk of primiparous and multiparous**

<table>
<thead>
<tr>
<th>Time</th>
<th>Primiparous (mg/liter)</th>
<th>Multiparous (mg/liter)</th>
<th>Primiparous (mg/liter)</th>
<th>Multiparous (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostrum</td>
<td>10.73±1.16</td>
<td>10.24±4.57</td>
<td>0.49±0.20</td>
<td>0.43±0.24</td>
</tr>
<tr>
<td>1 week</td>
<td>4.44±1.62</td>
<td>4.65±3.47</td>
<td>0.47±0.15</td>
<td>0.44±0.14</td>
</tr>
<tr>
<td>1 month</td>
<td>2.80±1.11</td>
<td>2.60±1.00</td>
<td>0.45±0.09</td>
<td>0.43±0.10</td>
</tr>
<tr>
<td>3 months</td>
<td>1.53±0.90</td>
<td>0.96±0.48</td>
<td>0.31±0.11</td>
<td>0.29±0.08</td>
</tr>
<tr>
<td>5 months</td>
<td>1.28±0.63</td>
<td>0.96±0.34</td>
<td>0.23±0.12</td>
<td>0.21±0.06</td>
</tr>
</tbody>
</table>

Mean±s.d.

**TABLE 3. Serum zinc and copper contents in 44 lactating women and 19 non-lactating control women**

<table>
<thead>
<tr>
<th></th>
<th>Zinc (µg/ml)</th>
<th>Copper (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactating women</td>
<td>0.76±0.13</td>
<td>1.29±0.18</td>
</tr>
<tr>
<td>Non-lactating women</td>
<td>0.85±0.09</td>
<td>1.08±0.11</td>
</tr>
</tbody>
</table>

Mean±s.d. $p<0.01$ $p<0.005$
et al. showed that higher contents of zinc and copper were found in breast milk of older women or of multiparous women than those of young or primiparous women, respectively (Piccano and Guthrie 1976). Our study could not confirm these observations. In addition, analysis at 3 months of lactation indicated that milk zinc contents decreased as years of maternal age increased.

In our observation the declining patterns of both trace minerals in breast milk were somewhat different. It was observed that zinc content declined much faster than copper content. Vaughan et al. (1979) also reported that the zinc declined appreciably but that the copper declined moderately. There are several possibilities to explain these findings, one of which although the detail is still obscure, may be due to different regulations of the transport mechanisms for each of the trace minerals from maternal serum to milk. In some animals, this mechanism is defined genetically (Piletz and Ganschow 1978). Content of each mineral in the maternal body might be another factor. Significantly lower zinc content and higher copper content were found in the serum of lactating women, when compared with non-lactating control women. It is known that copper levels rise during the pregnancy, and fall to normal values by approximately 6 weeks after delivery (Shaw 1980). However, no significant correlation in zinc or copper content was observed between milk and serum samples, in agreement with the previous findings by Rajalakshmi and Srikantia (1980). Similarly, zinc and copper levels of maternal hair samples showed no correlation with those of the breast milk samples. Vaughan et al. (1979) reported similar findings, although the number of their investigated samples was small. We believe that the correlation between these parameters should be evaluated further in the subjects covering malnutrition stage to normal stage, before a final conclusion is drawn.

In the present study, 3 of 64 cases had the levels of hair zinc content below 100 µg/g which will be implicated as zinc deficiency (Committee on Nutrition 1978), and the milk zinc levels in these cases were the lowest levels of the tested samples. It was shown that the amount of daily zinc intake did not affect the zinc content of breast milk (Karmarkar and Ramakrishnan 1960; Vuori et al. 1980), while in animal experiments, milk zinc content can be raised by increasing the zinc intake (Underwood 1971a, b). Thus, supplementation of zinc to maternal diet seemed to be useful in lactating women, especially in cases with zinc deficiency.

The recommendation of WHO Expert Committee on Trace elements in Human Nutrition (1973) proposed that infants less than 6 months of age require approximately 0.5 mg/kg per day of zinc and 0.08 mg/kg per day of copper. These recommended allowances correspond to a level of 3 to 5 mg/liter of zinc and a level of 0.4 mg/liter of copper in milk. The calculated daily intakes of zinc and copper, based on mean intakes of breast milk in Japan (Inoue 1960) (865 ml at 1 month, 845 ml at 3 months, 885 ml at 5 months) showed the followings: zinc 0.46, 0.16 and 0.13 mg/kg per day at 1, 3 and 5 months, respectively, and copper 0.08, 0.04, 0.03 mg/kg per day at 1, 3 and 5 months, respectively. The growth and clinical conditions of these infants were satisfactory, suggesting that zinc and
copper intakes seem to fit these requirement of Japanese infants though further assessment is necessary. Recently, citrate or picolinic acid in breast milk was shown to be zine-binding ligand which enhanced zinc absorption through the intestine (Evans and Johnson 1980; Hurley and Lönnerdal 1981). Thus, the daily requirement of trace elements might be considered on the basis of bioavailability of these elements.

References

