Human milk folate and folate status in lactating mothers and their infants

Tsunenobu Tamura, M.D., Yohzo Yoshimura, M.D., and Tsuneo Arakawa, M.D.

ABSTRACT Plasma and red blood cell folate levels of healthy, well-nourished lactating mothers were measured. Folate levels in their breast-fed infants were significantly higher than in the mothers. No abnormal hematological findings were observed in either mothers or infants. Folate levels in breast milk and in the infants’ plasma were significantly correlated. The mean breast-milk folate level was 141.4 ng/ml. The total daily folate intake for breast-fed infants was assessed at 14 to 25 μg/kg body weight. The plasma and red blood cell folate levels of the lactating mothers were significantly increased after oral administration of 1 mg pteroylmonoglutamate daily for 4 weeks. However, milk folate levels did not change.

Many studies have suggested that critical folate deficiencies occur in lactating mothers. Also, macrocytic anemia or megaloblastic anemia has been reported in malnourished lactating women (1–3). The folate deficiency of lactating mothers was successfully correlated by oral supplementation of folate (1). Metz (3) reported that the smallest dose of folate that produces hematological response in lactating mothers with folate deficiency is higher than that in nonlactating patients (3). In breast-fed infants, however, the incidence of megaloblastic anemia due to folate deficiency has been reported to be extremely rare. Folate levels in blood from breast-fed infants were higher than those in artificially-fed infants (4). This fact may indicate that breast milk is the best source of folate for young infants.

This study was carried out to investigate the effects of lactation on the folate status of lactating mothers and the relationship between folate content in mother’s milk and blood folate levels of their breast-fed infants. The recommended allowances of folate in infants were estimated from their daily folate intake based on the milk folate levels and amount of milk consumed.

Materials and methods

Subjects

Healthy, breast-fed infants (3 to 25 weeks old, weighing 6.32 ± 1.09 kg; mean ±SD, n = 25) were chosen from babies receiving regular monthly checkups at the pediatric clinic of a hospital located in the northern part of Japan. Their mothers were apparently healthy and had no history of any serious diseases at the time of this study. All subjects belonged to the same socioeconomic group and were from one geographic area. The selection of the subjects was based on the agreement and cooperation of their mothers. After the purpose and methods of the present study were explained to the mothers, consent forms were obtained. During the study, mothers were asked to continue with their normal dietary habits. However, dietary histories were not taken.

Collection of samples

Blood samples were obtained from both mothers and infants in early afternoon (2:00 to 3:00 PM). Subsequently, milk samples (approximately 10 ml) were collected by manual expression directly into test tubes containing ascorbic acid (5 to 10 mg) and were stored at −20 C until assayed. Milk samples were collected just before the babies were fed. Ramasastri (5) reported that milk folate levels (Lactobacillus casei, ATCC 7469, activity without pteroyl-polyglutamylhydrolyase (folate conjugase)) did not change during the course of emptying of the mammary glands (5).

Folate assay

Folate levels were determined by microbiological assay using L. casei (6). Plasma folate levels were measured.
In the present study, no abnormal hematological findings were observed in either lactating mothers or their infants (Table 1). As shown in Table 2, folate levels in the plasma and RBC of the mothers were within the normal range (8, 12). However, the plasma and RBC folate levels in their infants were much higher. Significant correlations between plasma and RBC folate levels were found in both mothers and infants with correlation coefficients of 0.69 (P < 0.01) and 0.48 (P < 0.01), respectively (Table 2).

In our study, we found that breast milk contained 141 ng/ml of “total folate” ranging from 62 to 280 ng/ml. “Free folate” levels, calculated at the half-maximal point of the *L. casei* response curve (6) accounted for approximately 40% of total folate in breast milk. This finding indicates that polyglutamate forms of folate derivatives are present in human milk.

Ramasastri (5) reported that folate levels in breast milk increased with the progress of lactation. In the present study, however, no significant changes were found in folate levels during lactation from 3 to 25 weeks after parturition.

Plasma folate levels in infants were significantly correlated with the breast milk folate levels of their mothers (Fig. 1). This suggests that the plasma folate level of breast-fed infants, which is an index of their folate nutritional status, is directly proportional to the folate content of their mother’s milk.

### TABLE 1

Hematological findings in lactating mothers and breast-fed infants

<table>
<thead>
<tr>
<th>Hemoglobin</th>
<th>Mean corpuscular volume</th>
<th>Lob average of neutrophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/100 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mothers</td>
<td>12.5 ± 1.1 (15)</td>
<td>94.0 ± 5.3 (15)</td>
</tr>
<tr>
<td>Infants</td>
<td>12.3 ± 1.3 (30)</td>
<td>92.3 ± 8.9 (30)</td>
</tr>
</tbody>
</table>

* The number of nuclear lobes in each of 100 neutrophils, stained with Wright-Giemsa, was counted and expressed as the average number of lobes per cell.

* Mean ± SD (number of cases).

### TABLE 2

Folate status in lactating mothers and breast-fed infants

<table>
<thead>
<tr>
<th></th>
<th>Plasma (ng/ml)</th>
<th>Red blood cells (ng/ml)</th>
<th>Milk* (J. casei activity ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mothers</td>
<td>5.9 ± 3.5 (39)</td>
<td>232.7 ± 83.6 (39)</td>
<td>141.4 ± 47.9 (39)</td>
</tr>
<tr>
<td>Infants</td>
<td>29.0 ± 13.6 (25)</td>
<td>429.1 ± 185.5 (25)</td>
<td></td>
</tr>
</tbody>
</table>

* L. casei activity after conjugase treatment.

Mean ± SD (number of cases).

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**Folic acid administration**

Sixteen lactating mothers were given folic acid (pteroylmonoglutamate, PteGlu, 1 mg per day) for 4 weeks after the collection of the first samples. The second samples were collected at the end of this administration period. Some mothers failed to take the folic acid tablets every day. However, the minimal amount of folic acid taken by any mother was 15 mg, and more than 90% of them ingested 23 to 28 mg folic acid in the 4-week period.

### Results

In the present study, no abnormal hematological findings were observed in either lactating mothers or their infants (Table 1). As shown in Table 2, folate levels in the plasma and RBC of the mothers were within the normal range (8, 12). However, the plasma and RBC folate levels in their infants were much higher. Significant correlations between plasma and RBC folate levels were found in both mothers and infants with correlation coefficients of 0.69 (P < 0.01) and 0.48 (P < 0.01), respectively (Table 2).

In our study, we found that breast milk contained 141 ng/ml of “total folate” ranging from 62 to 280 ng/ml. “Free folate” levels, calculated at the half-maximal point of the *L. casei* response curve (6) accounted for approximately 40% of total folate in breast milk. This finding indicates that polyglutamate forms of folate derivatives are present in human milk.

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TABLE 3
Plasma, RBC, and milk folate levels in lactating mothers before and after folate administration

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Red blood cells*</th>
<th>Milk*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before PteGlu...</td>
<td>6.0 ± 2.0 (16)</td>
<td>250.8 ± 85.2 (16)</td>
<td>130.2 ± 45.9 (16)</td>
</tr>
<tr>
<td>After PteGlu...</td>
<td>41.8 ± 53.8 (15)</td>
<td>389.9 ± 85.5 (15)</td>
<td>136.6 ± 41.2 (16)</td>
</tr>
</tbody>
</table>

* L. casei activity after conjugase treatment. ^ Oral administration of pteroylmonoglutamate (1 mg/day) for 4 weeks. Mean ± SD (number of cases).

In the present study, the daily folate intake for healthy infants can be calculated to be around 14 to 25 μg/kg body weight, assuming an infant consumption of 100 to 180 ml breast milk per kilogram daily. This would indicate that the average daily intake of folate in breast-fed infants ranges from 57 to 156 μg/day, since the mean body weight of infants was 6.32 kg.

Synthetic PteGlu (1 mg per day) was given orally for 4 weeks to sixteen random lactating mothers. After administration of PteGlu, maternal plasma, RBC, and milk folate levels were estimated and lobe averages of neutrophils were counted. As shown in Table 3, there were significant increases in plasma and RBC folate levels, but not in milk folate levels (P > 0.6). No significant changes in the lobe averages were found. Also, no significant correlations were found between breast milk folate levels and plasma or RBC folate levels from lactating mothers before and after PteGlu administration.

Discussion

As reviewed by Metz (3), macrocytic anemia was not infrequently found in lactating mothers, and this was considered to be due to lactation which might drain a significant amount of folate from maternal body stores. Abnormal hematological findings were not observed in the lactating mothers in this study, and their plasma and RBC folate levels were within the normal range (Table 2). In contrast, the plasma and RBC folate levels in their infants were higher than those in normal adults. These observations are identical with findings previously reported (13–18). Blood folate levels were high in early infancy, but after several months of life, had fallen to the normal range of adults (15, 17, 18).

It has been reported that free folate levels in breast milk range from 0.3 to 32 ng/ml (L. casei activity without conjugase treatment) (4, 5, 16, 19, 20), and total folate from 52 to 64 ng/ml (4, 21). Other workers, using Pediococcus cerevisiae and Streptococcus faecalis as test organisms, which do not respond to methylytetrahydrofolate, reported free folate levels of 7.3 and 0.71 ng/ml, respectively (4, 22). In our study, we found considerably higher levels of total folate in milk (Table 2), ranging from 62 to 280 ng/ml.

It is generally agreed that breast milk from healthy, well nourished mothers is the best food for young infants. It is also believed that the assessment of the recommended allowances of infants can be based on the composition of milk from well-nourished mothers (19). The values of folate intake (57 to 156 μg/day) presented in this report are slightly higher than the level of 50 μg/day for infants in Recommended Dietary Allowances (23), and in other reports (4, 21, 24–28). The requirement of folate, as compared with pure folic acid PteGlu, for healthy infants remains undetermined. It could be suggested that the daily nutritional requirement of pure folic acid for healthy infants is lower than the values presented in this study, since the availability of breast milk folate might be lower than synthetic folate compounds. The availability of food folate varies from food to food (11) and the availability of breast milk folate for infants is not known. Further studies are needed to investigate the availability of milk folate, with special reference to folate-binding protein(s) that might have a considerable influence on the vitamin economy and on the composition of the intestinal flora (4, 29).

Metz et al. (30) reported that there were appreciable increases in breast milk folate concentrations (less than 5 ng/ml to more than 60 ng/ml: L. casei activity without conjugase treatment) in two lactating patients with megaloblastic anemia, after 4 days of oral supplementation with 100 or 200 μg PteGlu daily (30). A possible reason for this...
large increase in milk folate is that the mothers were extremely folate deficient at the beginning of the study, resulting in abnormally low folate concentrations in their milk. These authors mentioned that in lactating mothers with folate deficiency, orally administered PteGlu might be taken up by the mammary glands in preference to hematopoietic system.

In the present study, we observed that there was no significant increase in breast milk folate levels of the lactating mothers after the oral administration of 1 mg PteGlu per day for 4 weeks. This might be due to the fact that the folate nutritional status in the lactating mothers was sufficient to maintain their breast milk folate levels before folate supplementation. Metz et al. (30) reported that milk folate levels increased before the hematological responses occurred. Based on the results of our study and that of Metz et al. (30), it appears that a regulatory mechanism exists to maintain folate levels in human milk. Folate-binding protein in milk may play a role in this regulation, but it is unlikely to be the whole answer. Ford et al. (31) reported that the folate content of goat milk was not wholly explained by its folate-binding capacity. Further studies are needed to elucidate the role of folate-binding protein(s) in breast milk, in particular its influence on the availability of milk folate and its possible role in the maintenance of constant milk folate levels.

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References

FOLATE STATUS IN LACTATING MOTHERS AND INFANTS