Maternal folate status during extended lactation and the effect of supplemental folic acid

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ABSTRACT

Background: Folate requirements during lactation are not well established.

Objective: We assessed the effects of dietary and supplemental folate intakes during extended lactation.

Design: Lactating women (n = 42) were enrolled in a double-blind, randomized, longitudinal supplementation trial and received either 0 or 1 mg folic acid/d. At 3 and 6 mo postpartum, maternal folate status was assessed by measuring erythrocyte, plasma, milk, and dietary folate concentrations; plasma homocysteine; and hematologic indexes. Infant anthropometric measures of growth, milk intake, and folate intake were also assessed.

Results: In supplemented women, values at 6 mo for erythrocyte and milk folate concentrations and for plasma homocysteine were not significantly different from those at 3 mo. In supplemented women compared with unsupplemented women at 6 mo, values for erythrocyte folate (840 compared with 667 nmol/L; \( P < 0.05 \)), hemoglobin (140 compared with 134 g/L; \( P < 0.02 \)), and hematocrit (0.41 compared with 0.39; \( P < 0.02 \)) were higher and values for reticulocytes were lower. In unsupplemented women, milk folate declined from 224 to 187 nmol/L (99 to 82 ng/mL), whereas plasma homocysteine increased from 6.7 to 7.4 \( \mu \)mol/L. Dietary folate intake was not significantly different between groups (380 ± 19 \( \mu \)g/d) and at 6 mo was correlated with plasma homocysteine in unsupplemented women (\( r = -0.53, P < 0.01 \)) and with plasma folate in supplemented women (\( r = 0.49, P < 0.02 \)).

Conclusions: A dietary folate intake of \( \approx 380 \) \( \mu \)g/d may not be sufficient to prevent mobilization of maternal folate stores during lactation.

Key Words: Lactation, plasma folate, erythrocyte folate, milk folate, dietary folate intake, plasma homocysteine, women

INTRODUCTION

The B vitamin folate and its coenzymatic forms are essential for one-carbon transfer reactions, which are underlying events for protein, DNA, and RNA biosynthesis. Folate requirements are greatest during periods of growth, development, and reproduction but may not always be met because folate deficiency occurs frequently during these nutritionally vulnerable stages of life. It is estimated that, globally, up to one-third of pregnant and lactating women have some degree of folate undernutrition (1). In the United States alone, \( \approx 15\% \) of women show signs of suboptimal folate status as evidenced by low serum and erythrocyte folate concentrations (<6.8 and <340 nmol/L, respectively) (2).

Substantial evidence now exists establishing a relation between enhanced folate intake during the periconceptual period and the prevention of neural tube defects (3, 4). Neural tube defects are among the most common birth defects in the United States and affect \( \approx 1 \) in 1000 infants (5). Compromised maternal folate status during pregnancy is also linked to low birth weight (6) and abruptio placentae (7) and to disorders that affect maternal health. Low folate status is associated with cellular dysplasia, often the predecessor of cancerous lesions in the epithelial layer of the cervix and colon (8), and elevated plasma homocysteine, a functional index of diminished folate status that is an independent risk factor for cardiovascular disease (9).

Although the requirement for folate during pregnancy has been studied extensively (10), folate needs for sustaining lactation are less well established (11). Most of our knowledge of the folate requirement of lactating women has been obtained indirectly from measurements of the amount of folate secreted in milk and from estimated maintenance requirements of the mother, which are assumed to be similar to those of nonreproducing women. Functional or health outcomes of various folate intakes during lactation are rarely examined (12).

A review of the literature indicates that apparently healthy women can become folate depleted in the early postpartum period (13). Milk folate content can be maintained at a level that prevents the development of folate inadequacy in exclusively breast-fed infants, but often at the expense of maternal folate stores. During lactation, folate is preferentially taken up by actively secreting mammary glands, and only in the case of frank maternal folate deficiency is milk folate reported to decline to critically low concentrations (14).
The incremental folate need for milk secretion was previously based on the assumption that the folate content of human milk averages 113 nmol/L (15); recent evidence from our laboratory indicates that this value was underestimated (16). After trienzyme treatment, typical folate concentrations of human milk range from 159 to 227 nmol/L (70–100 ng/mL) and average 195 nmol/L (86 ng/mL).

In this investigation, we studied women longitudinally during extended lactation and provided 0 or 1 mg folic acid (pteroylglutamic acid) daily in a double-blind, randomized fashion. Maternal folate status was assessed by using dietary, hematologic, and biochemical indexes. Measures of milk intake and infant growth were used as indexes of lactational performance.

SUBJECTS AND METHODS

Subjects

Women in the postpartum period living in and around State College, PA, were identified through newspaper birth announcements and a local lactation consultant. Potential subjects were sent a letter of invitation and contacted by telephone at 1–2 mo postpartum. After the purposes and procedures of the study were explained, women who agreed to participate with their infants were enrolled if they met the following eligibility criteria: apparently healthy, nonsmoking, no pregnancy complications, infant born at full term (37–40 wk), successfully nursing and planning to breastfeed, and a willingness to comply with the experimental protocol. The use of human subjects in this investigation was approved and traceptives, and willing to comply with the experimental protocol.

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Preliminary study

Because we were unaware of any reported values for plasma homocysteine concentrations of lactating women, before the start of the double-blind study, 10 women were recruited to establish normative values for plasma homocysteine under conditions of nutritional adequacy. These subjects were given a multivitamin and mineral supplement (Materna; Lederle Laboratories, Pearl River, NY) providing 12 µg vitamin B-12, 10 mg vitamin B-6, and 1 mg folic acid/d starting at 3 mo postpartum for 3 mo. A multivitamin supplement was chosen because, in addition to folate, vitamins B-6 and B-12 are known to influence plasma homocysteine concentrations (17).

Experimental design

A double-blind, randomized, longitudinal supplementation trial with 42 lactating women was conducted. Assignment of subjects to groups was balanced for parity. All subjects received a multivitamin and mineral supplement (Materna; Lederle Laboratories, Pearl River, NY) providing 12 µg vitamin B-12, 10 mg vitamin B-6, and 1 mg folic acid/d or a placebo tablet containing 0 mg folic acid/d (West-ward Pharmaceutical Corporation, Eatontown, NJ). The multivitamin and mineral supplement provided 2500 IU vitamin A as retinyl palmitate, 60 mg vitamin C, 400 IU vitamin D as ergocalciferol, 30 IU vitamin E as all-rac-α tocopheryl acetate, 1.5 mg thiamine, 1.7 mg riboflavin, 20 mg niacinamide, 2 mg vitamin B-6, 6 µg vitamin B-12, 300 µg biotin, 10 mg pantothenic acid, 9 mg Fe, 150 µg I, 3 mg Zn, 2.5 mg Mn, 25 µg Cr, and 25 µg Mo. Folic acid tablets were indistinguishable from placebo tablets. Multivitamin and mineral and folic acid or placebo preparations were provided in monthly allotments for the purpose of monitoring subject compliance. Compliance was defined as successfully taking 80% of the monthly allotment.

At 3 mo postpartum (baseline, before the start of supplementation) and at 6 mo postpartum (after 3 mo of supplementation), samples of breast milk and blood were collected and maternal diets were assessed. Measures of infant growth (weight, recumbent length, and head circumference) and milk intake were collected concurrently at 3 and 6 mo postpartum. All biological samples were handled under gold light to prevent photooxidization of folate and were stored at −70°C until analyzed.

Methods

Blood analyses

About 4 mL blood was drawn into tubes containing EDTA. A complete blood count and a differential smear were performed by using an electronic hematology analyzer (Max M model; Coulter Corporation, Miami). Measurements of hemoglobin, hematocrit, mean corpuscular volume, neutrophil hypersegmentation, and reticulocytes were included in the hematologic assessment. Neutrophil hypersegmentation was defined as a neutrophil lobe count >3.5. Hypersegmentation of neutrophils is a functional measure of abnormal folate metabolism (18). Portions of whole blood were frozen with 0.1 mol phosphate buffer/L containing 0.05 mol ascorbate/L for erythrocyte folate analysis. Plasma was separated from the remaining blood and frozen for folate and homocysteine analyses. Plasma and erythrocyte folate were measured by a microbiological assay with Lactobacillus casei (ATCC 7469) as the test organism (19). Plasma homocysteine was measured by HPLC according to the method of Araki and Sako (20), with some modifications. To measure non-protein-bound homocysteine, samples were mixed with 0.6 mol perchloric acid/L and then centrifuged at 1000 × g for 10 min at room temperature to precipitate plasma proteins.

Milk folate analysis

Complete breast expression was used to collect milk samples (15–30 mL) by manual means or with the assistance of a mechanical pump (Marshall Baby Care Products, Lincolnshire, IL; Ross Laboratories, Columbus, OH; or Evenflo Products, Ravenna, OH). Samples were stored with ascorbate to achieve a final concentration of 0.05 mol ascorbate/L. Data from our laboratory showed an 85% increase in measurable human milk folate after samples are treated with α-amylase (EC 3.2.1.1) and mycolysin (EC 3.4.24.31) in addition to folate conjugase (γ-Glu-X carboxypeptidase, EC 3.4.19.9) (16). Trienzyme treatment is now recognized for its merit in enhancing the measurement of folate in foods (21–23), but had not been previously applied to human milk samples. Before microbiological assays were performed, milk samples were incubated with α-amylase (type X-A; Sigma Chemical Co, St Louis), mycolysin (type XIV), and rat serum folate conjugase (Harlan Bioproducts, Indianapolis) at 37°C in a stepwise procedure for 4, 8, and 3 h, respectively. Milk samples were heat-treated in 0.1 mol phosphate buffer/L containing 0.05 mol ascorbate/L, pH 4.1, by boiling at 100°C for 5 min. The milk folate content was measured in a microbiological assay with L. casei as the test organism (19).
Dietary folate intake

Before 2-d food records were collected, subjects received instruction in accurately describing and quantifying their usual intake. Participants were instructed to use a 2-dimensional Food Portion Visual (Nutrition Consulting Enterprises, Framingham, MA) to aid in estimations of portion sizes. Dietary data were analyzed at the Diet Assessment Center at the Department of Nutrition, Pennsylvania State University. Nutrient intakes were tabulated by using NUTRITION DATA SYSTEM software (nutrient database version 24, food database version 9A; Nutrition Coordinating Center, University of Minnesota, Minneapolis). Folate intake was expressed as a 2-d average of recorded dietary intake at 3 and 6 mo postpartum. Complete nutrient profiles are reported elsewhere (24); folate intakes are reported here according to group assignment.

Anthropometry

Weight, recumbent length, and head circumference of infants were measured by using an electronic balance (Detecto Scale Company, Webb City, MO), a pediatric length-measuring board (Eillard Instrument Ltd, Seattle), and measuring tape (Ross Laboratories), respectively. Measures of infant growth were used as objective indexes of successful lactation. Self-reported heights and weights of mothers were obtained at entry to the study.

Infant milk and folate intakes

The daily milk intake of infants was measured by 24-h test weighing. Mothers were provided with an electronic balance (Detecto Scale Company) and instructed in proper weighing procedures. Infants were weighed before and after each feeding for 3 consecutive days. Milk intake was expressed as a 3-d average. With the introduction of foods other than breast milk, food records were used to assess infant nutrient intakes. By using data on milk intake and corresponding folate content, infant folate intakes were calculated.

Statistical analyses

SAS for WINDOWS (version 6.12, 1996; SAS Institute Inc, Cary, NC) was used for data tabulation and statistical analyses (25). Group differences were analyzed by analysis of variance (ANOVA) and covariance (ANCOVA) with repeated measures. Group (plus or minus folic acid) and time (3 and 6 mo) were treated as main effects, with a group by time interaction. Prenatal supplementation and parity were entered as covariates. One-way ANOVA was used to assess changes in biochemical and hematologic indexes and dietary folate intake within groups over time and between groups at one time point. Correlation analyses were used to determine associations among maternal indexes of folate status, dietary folate, supplemental folic acid, parity, and prenatal supplementation. A P value <0.05 was chosen as the level of significance. All values in the text, tables, and figures are presented as means ± SEMs.

RESULTS

Subject characteristics

Double-blind, longitudinal study

Characteristics of subjects participating in the double-blind, longitudinal study are presented in Table 1. The subjects’ ages ranged from 26 to 42 y (x̄: 34 y). Mean maternal weight (67 ± 3 kg), body mass index (BMI; in kg/m^2$: 25 ± 1), and parity (parity = 2) were not significantly different between groups, nor was folate intake from prenatal supplements, which averaged 0.9 mg/d. Of the 42 women studied, all were white and only 11 did not take prenatal supplements during pregnancy and in the early postpartum period before enrollment. Prenatal supplements used by women included Prenatal Plus (Rugby, Norcross, GA), Zenate (Solvay Pharmaceuticals, Marietta, GA), StuartNatal (Stuart, Atlanta), and Prenate-90 (Bock Pharmaceutal, St Louis), all of which provided 1 mg folic acid/d, and a variety of generic preparations that provided between 0.4 and 0.8 mg folic acid/d. Women enrolled were fairly well educated (16 years) and were from middle- to high-income households as indicated by a mean socioeconomic score of 72 (26).

Preliminary study

Characteristics of the lactating women (n = 10) studied to determine normative plasma homocysteine values were not significantly different from those of the women in the double-blind study. Mean maternal age was 32 y, self-reported maternal BMI was 25 ± 2, mean parity was 2, and mean socioeconomic score was 73. All women in the preliminary study took prenatal vitamin and mineral supplements that provided 1 mg folic acid/d during pregnancy and continued taking them until they were switched to a supplement provided by us at baseline (3 mo). Plasma folate (34.7 ± 3.8 and 29.6 ± 3.8 nmol/L) and erythrocyte folate (1025.7 ± 74 and 907.3 ± 75.3 nmol/L) concentrations of these women were not significantly different at 3 and 6 mo postpartum, respectively. Plasma homocysteine concentrations in these lactating women ranged from 4.1 to 11.9 μmol/L and averaged 7.4 ± 0.8 μmol/L at 3 mo and 7.1 ± 0.6 μmol/L at 6 mo. All values were below the cutoff of 15 μmol/L considered to be normal and well within the reported range established for the adult population (5–12 μmol/L) (27). Dietary folate intake from self-selected diets (430 ± 73 μg/d) was not significantly different at 3 and 6 mo.

Maternal measures

All measures used to assess the effect of enhanced folate intake during lactation are presented in Table 2. Baseline measures were made at 3 mo postpartum and after 3 mo of supplementation with

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>Selected characteristics at 3 mo postpartum (baseline) of lactating women receiving 0 or 1 mg supplemental folic acid/d in a double-blind longitudinal study^2^</td>
</tr>
<tr>
<td>Daily folic acid supplementation</td>
</tr>
<tr>
<td>Age (y)</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>BMI (kg/m^2$)</td>
</tr>
<tr>
<td>Parity</td>
</tr>
<tr>
<td>Education (y)</td>
</tr>
<tr>
<td>Socioeconomic status^2</td>
</tr>
<tr>
<td>Prenatal supplement (μg folic acid)</td>
</tr>
</tbody>
</table>

^2 ± SEM. There were no significant differences between groups. Socioeconomic status = (0.7 × education) + (0.4 × occupation), according to the method of Green (26). Scores in the 60s to 70s are associated with professional, technical, and managerial workers.
The mean folate intake from self-selected diets of the 42 lactating women studied was 390 ± 21 μg/d at 3 mo and 372 ± 31 μg/d at 6 mo; the overall mean was 380 ± 19 μg/d (Table 2). Dietary folate intakes were not significantly different between groups at either 3 or 6 mo. Folate intake at 6 mo was positively correlated with intakes at 3 mo (r = 0.69, P < 0.001).

Biochemical indexes

Supplemental folate had no significant effect on plasma folate values. Even though differences were not significantly different, mean plasma folate concentrations in the supplemented group (47.6 ± 6.5 nmol/L) were 10.8 nmol/L higher than those in the unsupplemented group (36.8 ± 4.2 nmol/L; Table 2). However, mean erythrocyte folate concentrations did respond significantly to folate supplementation. Values were higher at 6 mo in women receiving 1 mg supplemental folate/d than in unsupplemented women (P < 0.05). Both plasma and erythrocyte folate at 6 mo were positively associated with values at 3 mo (r = 0.35, P < 0.02, and r = 0.45, P < 0.01, respectively). Plasma folate was not associated with dietary folate at 3 mo but was at 6 mo (r = 0.38, P < 0.01).

In all but 2 women, values for plasma homocysteine (Table 2) were similar to values found in the preliminary study and well below the cutoff for hyperhomocysteinemia (< 15 μmol/L [28]). The 2 elevated values for plasma homocysteine (15.5 and 18.5 μmol/L) were noted at 6 mo in women who were receiving supplemental folate. Because all women also received 2 mg vitamin B-6 and 6 μg vitamin B-12, these elevated values were unrelated to vitamin intake. A time effect was noted for plasma homocysteine (P < 0.05). Within-group analysis showed that the increase in plasma homocysteine from 3 to 6 mo was significant in the unsupplemented group (P < 0.05), but not in the folate-supplemented group. Plasma homocysteine values at 6 mo were associated with values at 3 mo in both groups (r = 0.52, P < 0.01). In folate-supplemented women only, plasma homocysteine was inversely related to plasma folate at 6 mo (r = −0.53, P < 0.01). There also was an inverse relation between plasma homocysteine and dietary folate intake at 6 mo in women not receiving supplemental folate (r = −0.53, P < 0.01; Figure 1).

Hematologic indexes

A group effect for hemoglobin concentration was noted (P < 0.05; Table 2). At 6 mo, hemoglobin was significantly higher in folate-supplemented women than in unsupplemented women (P < 0.02). Repeated-measures ANOVA suggested a nonsignificant group by time interaction (P < 0.09) for reticulocytes and a nonsignificant group effect (P < 0.07) for hematocrit. The value for reticulocytes in both groups of women was within the normal range (0.005–0.015), but decreased in folate-supplemented women at 6 mo (P < 0.05) (29). Additionally, hematocrit values were higher in folate-supplemented women than in unsupplemented women at 6 mo (P < 0.02). No abnormal neutrophil lobe counts were noted at either 3 or 6 mo postpartum.
Milk folate

A time effect for milk folate concentrations was noted ($P < 0.03$; Table 2). In women not supplemented with folate, milk folate decreased significantly from 3 to 6 mo ($P < 0.02$). Folate supplementation, however, prevented a decline in milk folate from 3 to 6 mo postpartum. In folate-supplemented women only, milk folate was positively correlated with plasma homocysteine ($r = 0.71$, $P < 0.01$) and negatively correlated with plasma folate ($r = -0.52$, $P < 0.01$) at 6 mo.

Infant outcome measures

Anthropometry

Anthropometric indexes of growth (weight, length, and head circumference) indicated that all infants were growing normally (Table 3). All measures of infant growth increased significantly from 3 to 6 mo ($P < 0.001$) and there were no significant differences between groups. All infants were within the 5th and 95th National Center for Health Statistics (30) percentiles for weight and head circumference at both 3 and 6 mo. Nine infants were just above the 95th percentile and 1 was just below the 5th percentile for length at 3 mo; 4 infants were above the 95th percentile for length at 6 mo.

Milk and dietary folate intakes

Mean milk intake measured over 3 d was not significantly different between infants of mothers receiving supplemental folic acid and infants of mothers receiving placebo at both 3 and 6 mo. Milk intake was 679 ± 37 and 675 ± 47 mL/d at 3 and 6 mo, respectively. At 3 mo, all but 2 infants were exclusively breast-fed; at 6 mo, all but 8 infants were exclusively breast-fed. Foods other than breast milk furnished no more than 12% of daily energy intake.

The mean calculated folate intake of infants at 3 mo was 62 μg/d. At 6 mo, folate intake from breast milk alone was 55 μg/d and that from foods other than breast milk was 14 ± 3 μg/d. No group differences were noted in folate intakes of infants. Calculated intakes indicated that infants in this study were receiving on average the current recommended daily intake for folate (65 μg) at 3 mo and 80% of the recommended intake (80 μg) at 6 mo (31).

Discussion

Results from the present study clearly showed that dietary folate needs during lactation are far greater than estimated previously (15). Women in this study who consumed an average of 380 μg folate/d from foods displayed diminished biochemical indexes of maternal folate status: a decline in milk folate and an increase in plasma homocysteine from 3 to 6 mo postpartum. At 6 mo, lactating women not receiving supplemental folate also had lower values for hemoglobin, hematocrit, and erythrocyte folate and had a higher average value for reticulocytes than did women receiving 1 mg folate/d. These indexes of folate status either improved or remained relatively stable in women supplemented with folate. Although the level of supplementation used in this study did not allow us to determine the quantity of dietary folate needed to prevent mobilization of maternal stores, the data do indicate that a daily dietary folate intake of 380 μg is not always adequate for meeting the demands of lactation. Thus, the average need for folate during lactation must be > 380 μg/d.

Estimated average folate needs during lactation can be calculated by using data from the present study in conjunction with published data. The previous recommended dietary allowance for folate during lactation was based on the estimated requirement of nonpregnant, nonlactating women (180 μg/d), with an additional 100 μg/d to cover losses due to milk secretion (15). That increment was based on the assumptions of a human milk folate content of 113 nmol/L (50 ng/mL) and an average daily milk production of 800 mL/d during established lactation. It is now fairly well established that folate needs for nonreproducing women are similarly greater than estimated previously. Both Sauberlich et al (32) and O’Keefe et al (33) investigated folate requirements of nonreproducing women consuming diets with defined folate contents. These investigations estimated folate requirements to be 200–400 μg/d as assessed from changes in biochemical and functional indexes of folate status. Data from the present study show that the average folate content of human milk (195 nmol/L, or 86 ng/mL) is 72% greater than the value used previously and that corresponding folate losses due to milk secretion are also greater. From these data and by using the same assumptions used by the Food and Nutrition Board for food folate bioavailability (50%) and milk volume transferred to infants (780 mL) (31), we estimate the average requirement for folate during lactation to be ≈ 455 μg/d. This estimate assumes an average folate requirement for nonreproducing women of 320 μg/d and includes an increment of ≈ 135 μg/d for milk synthesis (780 mL/d × 86 μg/1000 mL × 100/50 = 135 μg/d). The recommended dietary allowance for folate during lactation was recently increased from 280 to 500 μg/d (31).

We observed a decline in mean milk folate content (from 224 to 187 nmol/L) from 3 to 6 mo in women ingesting 380 μg folate/d without supplemental folate, whereas women receiving supplemental folate maintained similar values at 3 and 6 mo (186 and 182 nmol/L). Folate in human milk is reported to remain stable or to increase as lactation progresses (34–37) and there is considerable variability in reported mean values for folate in human milk (50–319 nmol/L) (13). It is now well established that methodologic difficulties rather than differences among individuals are responsible for the wide range in values reported for milk folate (16, 38, 39). Previously, our laboratory reported that in the analysis of human milk folate, it is essential to preserve labile folates with ascorbate or another antioxidant, to heat samples to denature folate-binding proteins, and to treat samples

**TABLE 3**

Mean measures of infant growth at birth and at 3 and 6 mo of age

<table>
<thead>
<tr>
<th>Maternal daily folic acid supplementation</th>
<th>0 mg ($n = 21$)</th>
<th>1 mg ($n = 21$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>3.6 ± 0.1</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>53.6 ± 0.6</td>
<td>53.2 ± 0.6</td>
</tr>
<tr>
<td>3 mo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>6.7 ± 0.2</td>
<td>6.4 ± 0.1</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>61.6 ± 0.6</td>
<td>61.9 ± 0.5</td>
</tr>
<tr>
<td>Head circumference (mm)</td>
<td>41.6 ± 0.3</td>
<td>41.3 ± 0.2</td>
</tr>
<tr>
<td>6 mo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>8.0 ± 0.1</td>
<td>7.9 ± 0.2</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>67.6 ± 0.5</td>
<td>67.7 ± 0.5</td>
</tr>
<tr>
<td>Head circumference (mm)</td>
<td>44.3 ± 0.2</td>
<td>44.3 ± 0.3</td>
</tr>
</tbody>
</table>

7 ± SEM.

1 Infant measures as reported by mothers.

2 Gains in weight, length, and head circumference from 3 to 6 mo were significant for both groups, $P < 0.0001$. No significant differences between groups were noted.

$\text{SEM}$. The American Journal of Clinical Nutrition by guest on December 16, 2013
with folate conjugase to cleave glutamyl residues (38). We also applied 2 other enzymatic treatments [$\alpha$-amylase and mycolysin, which were recently shown to enhance measurable folate in other biological samples (21–23)] to our milk samples before they were analyzed by microbiological assays. Using this improved method, we observed an 85% increase in measurable human milk folate compared with that observed with treatment with folate conjugase alone (16).

Plasma homocysteine values for nearly all of the lactating women in this study were within the normal range reported for nonreproducing women (27). Nonetheless, plasma homocysteine increased significantly from 3 to 6 mo postpartum in women not receiving supplemental folate but remained stable in women receiving supplemental folate. There is general agreement in the literature that plasma homocysteine is negatively associated with dietary folate intake and plasma folate (33, 40–42). Plasma homocysteine was negatively associated with plasma folate only in the folate-supplemented group and with dietary folate only in the unsupplemented group at 6 mo.

Plasma folate concentrations did not change significantly from 3 to 6 mo postpartum in either supplemented or unsupplemented women. This may reflect the fact that all participants in this study had relatively high plasma folate concentrations at baseline (16–70 nmol/L). Ekelund (35) reported that length of lactation is inversely related to plasma folate concentrations. Folate supplementation during lactation is reported to have differing effects on plasma folate. Tamura et al. (43) reported plasma folate concentrations to be 7 times greater than at baseline after 4 wk of supplementation with 1 mg folate/d. Women in that study, however, were at different stages of lactation and had an average plasma folate concentration of 13 nmol/L before supplementation. Conversely, Thomas et al. (44) reported that plasma folate was similar between folate-supplemented (with 0.8 mg/d) and unsupplemented lactating women at 6 mo postpartum (29.5 nmol/L). In Finnish women receiving 0.1 mg folate/d, plasma folate increased from $\approx$16 to 20 nmol/L, yet up to 10% of those presumably well-nourished lactating women exhibited signs of folate inadequacy (plasma folate $\leq$7 nmol/L) from 1 to 6 mo postpartum (45).

All women in this study had erythrocyte folate values well above the value indicative of deficiency, $<340$ nmol/L (46). Erythrocyte folate concentrations were not significantly different between groups at 3 mo, but were elevated in supplemented compared with unsupplemented women at 6 mo. Erythrocyte folate is reported to decline during both pregnancy (47) and lactation in the early postpartum period (36, 47, 48). Because erythrocyte folate concentrations are thought to parallel liver folate stores, the diminished values observed in unsupplemented women indicate that liver folate stores were mobilized to meet the nutritional demands of lactation. The extent to which maternal folate stores can continue to compensate for dietary deficits during lactation is not known. The importance of such information is stressed considering that current recommendations are for continued breast-feeding for $\geq$12 mo (49) and that nutrition supplementation is not necessary during lactation (50).

Most unexpected were our findings of elevated hemoglobin and hematocrit concentrations in the folate-supplemented group at 6 mo. Consistent with these findings was a decline in reticulocytes in supplemented but not unsupplemented women from 3 to 6 mo. These patterns of change in hematologic indexes suggest that some degree of ineffective erythropoiesis was evident in women not receiving supplemental folate. The hematologic responses can be ascribed to folate because all women received a multivitamin and mineral supplement providing ample quantities of iron and vitamins B-6 and B-12.

With use of traditional assessment methods for lactational performance (infant anthropometric indexes, volume of milk ingested, and milk folate content), we judged women in the present investigation to be lactating successfully. All infants displayed weight gain within the 5th and 95th percentiles of the National Center for Health Statistics standards and showed no signs of growth faltering. Average milk intake of infants in the present study ($=675$ mL/d) was well within the expected range (51). Infants in this study received milk that furnished on average $80$–$100$% of the current recommended dietary allowance for folate (65 $\mu$g/d at 3 mo and $80$ $\mu$g/d at 6 mo). Although infants received apparently adequate amounts of folate and were growing within expected ranges, maternal folate status was deteriorating. Other investigations reported that maternal folate stores can be depleted to maintain milk folate content and that folate deficiency is precipitated by lactation (14).

In January 1998, mandatory folic acid grain-enrichment policies were initiated (52). Cereal grains fortified with 140 $\mu$g folic acid/100 g are predicted to increase dietary folate intakes by $=100$ $\mu$g/d for women of reproductive age (53). In the present study, lactating women obtained 30% of their total daily dietary folate from fortified, ready-to-eat cereals (54), which are folate sources of high bioavailability (55). However, current enrichment policies do not alter the amount of folate added to ready-to-eat cereals, so it is not certain whether and to what extent such policies will affect the folate intakes of lactating women. National data on distribution patterns of foods contributing folate to diets of lactating women are needed.

In summary, results from the present study showed that folate status diminished in lactating women consuming $=380$ $\mu$g dietary folate/d but that maternal folate status was preserved with 1 mg supplemental folate. Despite the reduction in folate status in lactating women, average milk folate contents were maintained at levels furnishing 100% and 80% of the current recommended dietary allowances for infants at 3 and 6 mo, respectively (31). These findings stress the importance of including maternal indexes of nutritional adequacy in addition to infant indexes when assessing lactational performance.

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