Concentrations of unmetabolized folic acid and primary folate forms in pregnant women at delivery and in umbilical cord blood

Rima Obeid, Mariz Kasoha, Susanne H Kirsch, Winfried Munz, and Wolfgang Herrmann

ABSTRACT

Background: The importance of unmetabolized folic acid in maternal and fetal blood is not known.

Objective: We investigated total folate, tetrahydrofolate (THF), 5-methyltetrahydrofolate (5-MTHF), formyl-THF, 5,10-methenylTHF, and folic acid concentrations in women and in umbilical cord blood at delivery.

Design: The study included 87 pregnant women and 29 cord blood samples, including 24 mother-infant pairs. We measured serum concentrations of folate forms by using ultraperformance liquid chromatography–tandem mass spectrometry.

Results: Pregnant women who received 400 μg folic acid daily (n = 25) had higher total folate (P = 0.041), 5-MTHF (P = 0.049), and formyl-THF (P < 0.001) concentrations and slightly higher THF (P = 0.093) concentrations than did nonsupplemented pregnant women (n = 61). We measured folic acid concentrations >0.20 nmol/L in 38 (44%) pregnant women and in 55% of the cord serum samples, but these measurements were not explained by maternal supplement use. Concentrations of folic acid were nonsignificantly higher in cord blood from supplemented women than in cord blood from nonsupplemented women (P = 0.154). Proportions of folic acid to total folate in cord serum did not differ according to maternal supplement use (0.54% compared with 0.43% in supplemented and nonsupplemented women, respectively). Concentrations of folic acid did not differ between maternal and cord serum. However, folic acid constituted a significantly lower proportion of total folate in cord serum than in maternal serum.

Conclusions: We detected unmetabolized folic acid in more than one-half of cord blood samples. Folic acid (400 μg/d) supplied during pregnancy is not likely to accumulate in the fetus, in contrast to 5-MTHF and THF, which accumulate in the fetus.

INTRODUCTION

Folate is an essential micronutrient during fetal development because of its role in transmethylation reactions and in de novo synthesis of DNA in growing cells. Low maternal folate status has been linked to birth defects such as neural tube defects (NTDs) (1). Supplementation with folic acid lowers serum concentrations of total homocysteine (tHcy) and protects against NTDs (2). 5-Methyltetrahydrofolate (5-MTHF) supplies a labile methyl group via methionine as S-adenosylmethionine. 5,10-Methylene tetrahydrofolate (5,10-methenylTHF) donates a methyl group for 2′-deoxyuridine-5′-monophosphate to form 2′-deoxycytidine-5′-monophosphate or is oxidized to 5,10-methenylTHF; this is further converted to 10-formyl-THF, which participates in purine synthesis. 5,10-Methylene tetrahydrofolate reductase (MTHFR) is a key enzyme that affects the flow of one-carbon units to their acceptors. The enzyme is subject to allosteric inhibition by S-adenosylmethionine (3). A common mutation in the gene encoding for MTHFR (C677T) results in reduced in vitro activity and can cause increased formylated folate (4) or elevated concentrations of tHcy when nutritional folate is insufficient (5).

The use of folic acid supplements is common during pregnancy. Despite the unequivocal success of folic acid in reducing NTD rates, several studies questioned the role of unmetabolized folic acid in blood (6–9). Folic acid supplementation in late pregnancy was related to asthma risk in children at 5 y of age (10). In the Norwegian Mother and Child Cohort Study, exposure to folic acid in the first trimester of pregnancy was associated with a slightly increased risk of respiratory illness in the first 18 mo after birth (11). Moreover, folic acid in the circulation was related to lower natural-killer cell cytotoxicity in elderly women (7). It was postulated that the presence of unmetabolized folic acid could affect the normal homeostatic regulation of folate (12). To our knowledge, no firm data are available to support the notion that folic acid in blood can cause harm. In a rat model, long-term supplementation with folic acid resulted in lower birth weights (13). However, these results cannot be extrapolated to humans because species differences in folic acid metabolism have recently been shown (14).

How maternal folate metabolism reflects fetal metabolism and how folic acid supplementation affects fetal folate have not been adequately investigated. The concentration of total folate is considerably higher in fetal than in maternal serum (15). The placenta seems to extract folate from the maternal circulation and concentrates it in the fetus (16, 17), probably via a carrier-mediated process (18). In addition, folic acid has been detected in cord blood and in formula-fed babies (19). Sweeney et al (20) reported detectable amounts of folic acid in 17 of 20 placental cord blood samples and 18 of the corresponding pregnant women at delivery. Variations in folic acid intake, absorbance, metab-

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olism, or polymorphisms in folate-metabolizing enzymes might affect the amount of free folic acid in blood. The role of supplement use and maternal folate status as determinants of folic acid and other folate forms in cord blood deserves further investigation. The aim of this study was to investigate primary folate forms in pregnant women and umbilical cord blood at birth. In addition, we tested a possible effect of 2 common polymorphisms in MTHFR (C677T) and reduced folate carrier (RFC-1 G80A) genes on folate distribution in pregnant women at delivery.

SUBJECTS AND METHODS

The study included 87 pregnant women who were randomly recruited from consecutive deliveries in the Department of Obstetrics and Gynecology at the University Hospital of Saarland. The study took place between June 2004 and January 2007. All women were free of chronic diseases. Mothers who expected infants with any type of congenital malformation, and mothers <17 y of age were not eligible for the study. Women with multiple pregnancies were also excluded. Premature birth or intrauterine growth retardation were not exclusion criteria for the study. Both vaginal and cesarean births were included.

Maternal anthropologic measures and data on weight increase and vitamin use during pregnancy, parity, and gravidity were obtained. Gestational age was defined on the basis of the last menstrual date and ultrasound examination. Clinical variables for newborns were also available (ie, weight, length, head circumference, blood gases, and venous and arterial blood pH) (Table 1). The study also included 29 samples of umbilical cord blood at birth. Mothers of 24 of the newborns also participated in the study. Furthermore, 25 nonpregnant women who were not taking any supplement containing folic acid were included in the study. The study was approved by the local ethical committee (Saarland, Germany), and all participants gave their informed consent to participate in the study.

Peripheral venous blood samples were obtained from mothers 1–12 h before birth. The mean (±SD) birth duration was 7.5 ± 4 h. Women were not allowed to eat or drink for at least the duration of the birth. A blood sample was collected from the umbilical vein immediately after expulsion of the placenta. Maternal and cord blood samples were collected in tubes without anticoagulant, left to clot for ≤45 min, and centrifuged at 2000 × g at 4°C. Serum was directly separated and stored at −70°C until analysis. Blood samples from pregnant and nonpregnant subjects were collected, separated, and stored under similar conditions.

Concentrations of tHcy were measured by gas chromatography-mass spectrometry as described elsewhere (21). Concentrations of primary folate forms were measured in serum on an Acquity Ultra Performance LC system (Waters Corporation, Milford, MA) coupled to a MicroMass Quattro Premier XE tandem quadrupole mass spectrometer (Waters Corporation) as previously reported (22). In brief, serum samples (250 μL) were incubated with 700 μL ammonium acetate buffer (200 mmol/L; pH 10). Then, 50 μL internal standard solution (1 μmol/L [13C5] 5-methylTHF, 0.5 μmol/L [13C8] folic acid, and 0.2 μmol/L [13C4]5-formylTHF) was added. Within ~15 min, a sample cleanup was performed on Oasis MAX solid-phase extraction columns (Waters Corporation) preconditioned with methanol followed by ammonium acetate buffer (pH 10) that contained 10 g ascorbic acid/L. The samples were loaded, and impurities were removed, by washing the columns with 5% aqueous NH4OH and methanol. Folate forms were eluted with methanol that contained 1% formic acid. The total folate concentration was calculated by adding the concentrations of the folate forms in the sample. The limit of detection (LOD) for folic acid concentrations defined as a signal-to-noise ratio ≥5 by using our ultraperformance liquid chromatography–tandem mass spectrometry method was 0.20 mmol/L. Free folic acid might be bound on plasma proteins. Therefore, we tested the effect of a longer incubation time (≤1 h) and acidic conditions (pH 4.5) during sample preparation on free folic acid concentrations at 2 amounts of free folic acid (high and low) (see supplemental Tables 1 and 2 under “Supplemental data” in the online issue). We observed slightly higher concentrations of folic acid in samples with a high folic acid content that were prepared at pH 4.5 or incubated for 1 h than in samples prepared at pH 10 or in samples separated without incubation, respectively. However, this was observed only in the higher range of free folic acid, which was not relevant in this study.

DNA was isolated from maternal and cord blood samples. MTHFR C677T and RFC-1 G80A polymorphisms were investigated by using primers and methods as previously described (23, 24).

Data analyses were performed with SPSS software (version 17.0; SPSS, Chicago, IL). A Kolmogorov–Smirnov test was used to check for normality. Folate concentrations were normally distributed except for maternal THF and folic acid. Results are presented as either medians (10th–90th percentiles) or as means (±SDs). In Tables 2–4, comparisons of medians of 2 groups—supplemented and nonsupplemented or pregnant and nonpregnant—were performed by using the Mann–Whitney U test. Possible differences in the means of different variables between maternal and cord serum were investigated by using a paired Student’s t test (Table 5). The parametric analysis of variance and Tamhane tests were applied for multiple comparisons of means after log transformation of skewed variables shown in Table 6. Chi-square tests were performed to test differences in categorical variables. Correlations between different variables were examined by the Spearman’s test. All

### Table 1

Main characteristics of pregnant women and newborns

<table>
<thead>
<tr>
<th>Category</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant women (n = 87)</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>30 (22–37)</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>39.0 (36.0–40.2)</td>
</tr>
<tr>
<td>Weight increase (kg)</td>
<td>13.0 (7.0–20.0)</td>
</tr>
<tr>
<td>Folic acid supplement [% (%)]</td>
<td>25 (29)</td>
</tr>
<tr>
<td>Newborns (n = 29)</td>
<td></td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3390 (2652–4000)</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>52 (48–55)</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>34.5 (32.0–36.5)</td>
</tr>
<tr>
<td>Mother supplemented with folic acid [% (%)]</td>
<td>10 (34)</td>
</tr>
</tbody>
</table>

1 Median; 10th–90th percentiles in parentheses (all such values).
2 Four hundred micrograms folic acid/d was consumed from the first month and throughout pregnancy.
5,10-MethenylTHF (nmol/L) 0.02 (0.0–0.13) 0.18 (0.05–0.33) 0.17 (0.01–0.34) b 0.20 (0.11–0.33) 0.145

Formyl-THF (nmol/L) 0.16 (0.05–0.35) a,b 0.16 (0.04–0.30) 0.13 (0.03–0.27) b 0.25 (0.08–0.38) a <0.001

THF (nmol/L) 2.1 (0.35–3.8) 1.8 (0.5–3.8) 1.6 (0.4–3.4) 2.2 (0.9–4.3) 0.093

5-MTHF (nmol/L) 15.8 (6.4–27.7) a,b 15.0 (4.0–41.9) 11.7 (3.9–44.5) b 24.7 (4.8–39.6) a 0.049

Folic acid (nmol/L) 0.10 (0.03–0.77) 0.15 (0.00–0.80) 0.13 (0.00–0.78) b 0.19 (0.00–0.91) 0.314

5-MTHF, 5-methyltetrahydrofolate; THF, tetrahydrofolate; tHcy, total homocysteine. Values with the same superscript letter were significantly different for comparisons between nonpregnant women and nonsupplemented or supplemented pregnant women (medians compared by using the Mann-Whitney U test). Supplements containing 400 μg folic acid/d were started in the first month and continued throughout pregnancy.

Nonpregnant women were not supplemented with folic acid.

One outlier was omitted.

Mann-Whitney U test was used to compare supplemented with nonsupplemented pregnant women, except where otherwise indicated.

Median; 10th–90th percentiles in parentheses (all such values).

Chi-square test was used to compare supplemented with nonsupplemented pregnant women.

tests were 2-sided, and P < 0.05 was considered statistically significant.

RESULTS

The main characteristics of the 87 pregnant women participating in the study and the 29 newborns for which we had umbilical cord blood are shown in Table 1. Twenty-five pregnant women used 400 μg folic acid/d throughout their pregnancy starting from the first month. Data sets from 24 mothers and their newborns were available.

Concentrations of folate forms in nonpregnant and pregnant women

Concentrations of folate forms and tHcy in nonpregnant and pregnant women according to maternal vitamin use are shown in Table 2. Pregnant women who took folic acid had higher total folate (P = 0.041), 5-MTHF (P = 0.049), THF (P = 0.093), and formyl-THF (P < 0.001) concentrations than did nonsupplemented pregnant women. Higher total serum folate and 5-MTHF concentrations in supplemented pregnant women were not associated with a significant difference in folic acid amounts compared with the concentrations in nonsupplemented pregnant women, which suggested that folic acid was sufficiently metabolized. Concentrations of folic acid were above the LOD (0.20 nmol/L) in 38 of the 87 pregnant women (43.6%), with no significant difference in the percentage of pregnant women with detectable folic acid in serum according to folic acid use.

The group of nonpregnant women had concentrations of total folate (median: 18.1 nmol/L) and 5-MTHF (median: 15.8 nmol/L) comparable with the concentrations in pregnant women who did not use folic acid but that were lower than the concentrations in pregnant women who used a supplement (Table 2). Of the nonpregnant women, 5 (20%) women had detectable concentrations of folic acid (≥0.20 nmol/L) and 2 women had folic acid concentrations >1.35 nmol/L, but none of the women were
using supplements that contained folic acid. Fewer nonpregnant women showed detectable folate acid concentrations in serum than did pregnant women (20.0% compared with 43.6%; \( P = 0.033 \), chi-square test).

### Concentrations of folate forms in umbilical cord blood

Concentrations of folate forms in umbilical cord serum samples did not significantly differ according to maternal folic acid use (Table 3). Furthermore, percentages of cord serum samples with detectable folate acid concentrations did not significantly differ according to maternal supplement use (52.6% compared with 60.0% for cord blood from nonsupplemented and supplemented mothers, respectively). The proportions of folic acid to total folate in cord serum did not differ according to maternal supplement usage (0.54% compared with 0.43% in supplemented and nonsupplemented women, respectively).

### Detectable compared with undetectable unmetabolized folic acid

We also divided subjects into those with circulating folic acid concentrations <0.20 or \( \geq 0.20 \text{ nmol/L} \) (Table 4). Women with detectable concentrations of folic acid in serum (\( n = 38 \)) were not more likely to be supplemented with folic acid than were women with folic acid below the LOD (\( n = 49 \)) (12 women compared with 13 women, respectively; \( P = 0.606 \); chi-square test). As shown in Table 4, concentrations of maternal total folate, 5-MTHF, and THF and cord serum 5-MTHF concentrations were significantly different between the groups with folic acid concentrations <0.20 or \( \geq 0.20 \text{ nmol/L} \). Of the available cord blood samples, significantly more samples from supplemented women showed folic acid concentrations \( \geq 0.20 \text{ nmol/L} \) compared with <0.20 nmol/L (Table 4).

### Concentrations of folate forms in cord serum compared with maternal serum

Results for the 24 mother-cord pairs are shown in Table 5. Total folate and 5-MTHF concentrations were higher in cord than in maternal serum. 5-MTHF constituted a higher proportion of total folate in cord than in maternal serum. THF concentrations tended to be higher in cord than in maternal serum. Concentrations of folic acid did not differ significantly between maternal and cord serum. However, folic acid constituted a significantly lower proportion of total folate in cord than in maternal serum (0.49% compared with 2.4%) (Table 5).

Concentrations of folic acid were positively correlated to 5-MTHF concentrations in maternal (\( r = 0.42, P < 0.001 \); Spearman’s \( r \)) and cord serum (\( r = 0.62, P < 0.001 \)). Correlations between folic acid and 5-MTHF in mothers and cord blood according to folic acid use are shown in Figure 1. Finally, concentrations of primary folate forms in maternal serum did not significantly correlate with concentrations of primary folate forms in cord serum. A significant positive correlation was shown between cord blood concentrations of folic acid and of formyl-THF (\( r = 0.582, P = 0.001; n = 29 \)) and 5-MTHF (\( r =

### TABLE 4

Concentrations of maternal and cord blood folate forms according to maternal folic acid supplement use 1

<table>
<thead>
<tr>
<th></th>
<th>Maternal folic acid &lt;0.20 nmol/L</th>
<th>Maternal folic acid ( \geq 0.20 \text{ nmol/L} )</th>
<th>( P^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>49</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Maternal total folate (nmol/L)</td>
<td>7.2 (5.5–13.3) 1</td>
<td>13.2 (6.2–28.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>Maternal 5-MTHF (nmol/L)</td>
<td>5.6 (3.9–12.2)</td>
<td>11.2 (4.5–25.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>Maternal THF (nmol/L)</td>
<td>1.0 (0.2–1.4)</td>
<td>1.3 (0.8–2.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Folic acid (nmol/L)</td>
<td>—</td>
<td>0.31 (0.22–0.61)</td>
<td>1</td>
</tr>
<tr>
<td>Supplemented folic acid [( n ) (%)]</td>
<td>13 (26.5)</td>
<td>12 (31.6)</td>
<td>0.604 4</td>
</tr>
<tr>
<td>No. of available cord blood samples</td>
<td>15</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Cord serum total folate (nmol/L)</td>
<td>10.1 (6.0–30.7)</td>
<td>35.7 (26.5–51.3)</td>
<td>0.035</td>
</tr>
<tr>
<td>Cord serum 5-MTHF (nmol/L)</td>
<td>8.9 (4.2–28.3)</td>
<td>32.7 (24.0–48.9)</td>
<td>0.033</td>
</tr>
<tr>
<td>Cord serum THF (nmol/L)</td>
<td>1.0 (0.5–2.0)</td>
<td>1.9 (1.5–2.5)</td>
<td>0.333</td>
</tr>
<tr>
<td>Folic acid (nmol/L)</td>
<td>0.01 (0.00–0.08)</td>
<td>0.21 (0.01–0.26)</td>
<td>0.083</td>
</tr>
<tr>
<td>Folic acid ( \geq 0.20 \text{ nmol/L} ) [( n ) (%)]</td>
<td>5 (33)</td>
<td>8 (89)</td>
<td>0.010 4</td>
</tr>
</tbody>
</table>

1 5-MTHF, 5-methyltetrahydrofolate; THF, tetrahydrofolate. The limit of detection for folic acid is 0.20 nmol/L (defined as a signal-to-noise ratio of 5).
2 Calculated by using the Mann-Whitney \( U \) test, except where otherwise indicated.
3 Median, 10th–90th percentiles in parentheses (all such values).
4 Calculated by using the chi-square test.

### TABLE 5

Concentrations of maternal and corresponding cord blood folate forms 1

<table>
<thead>
<tr>
<th></th>
<th>Pregnant women (( n = 24 ))</th>
<th>Cord blood (( n = 24 ))</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total folate (nmol/L)</td>
<td>18.0 ± 12.4</td>
<td>39.0 ± 22.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5-MTHF (nmol/L)</td>
<td>15.6 ± 11.5</td>
<td>35.8 ± 21.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>THF (nmol/L)</td>
<td>1.8 ± 1.2</td>
<td>2.6 ± 1.7</td>
<td>0.050</td>
</tr>
<tr>
<td>Formyl-THF (nmol/L)</td>
<td>0.17 ± 0.10</td>
<td>0.25 ± 0.21</td>
<td>0.087</td>
</tr>
<tr>
<td>5,10-MethenylTHF (nmol/L)</td>
<td>0.21 ± 0.13</td>
<td>0.20 ± 0.13</td>
<td>0.704</td>
</tr>
<tr>
<td>Folic acid (nmol/L)</td>
<td>0.30 ± 0.33</td>
<td>0.20 ± 0.18</td>
<td>0.165</td>
</tr>
<tr>
<td>5-MTHF (%)</td>
<td>82.5 ± 12.7</td>
<td>89.4 ± 6.5</td>
<td>0.034</td>
</tr>
<tr>
<td>THF (%)</td>
<td>11.9 ± 8.4</td>
<td>8.4 ± 5.6</td>
<td>0.143</td>
</tr>
<tr>
<td>Formyl-THF (%)</td>
<td>1.52 ± 1.34</td>
<td>0.80 ± 0.82</td>
<td>0.016</td>
</tr>
<tr>
<td>5,10-MethenylTHF (%)</td>
<td>1.67 ± 1.80</td>
<td>0.89 ± 1.3</td>
<td>0.107</td>
</tr>
<tr>
<td>Folic acid (%)</td>
<td>2.4 ± 3.7</td>
<td>0.49 ± 0.48</td>
<td>0.017</td>
</tr>
</tbody>
</table>

1 All values are means ± SDs. 5-MTHF, 5-methyltetrahydrofolate; THF, tetrahydrofolate. Folic acid supplement use was reported by 7 women. A paired \( t \) test was applied to compare mean concentrations between maternal and cord blood samples. Results were checked for normal distribution, and when this was not the case data were log-transformed to ensure a normal distribution before applying the \( t \) test.
0.620, $P < 0.001$; $n = 29$). Maternal folic acid concentrations were directly related to maternal 5-MTHF ($r = 0.423$, $P < 0.001$; $n = 87$) and THF concentrations ($r = 0.356$, $P = 0.001$; $n = 87$). Maternal and cord blood folate forms were not significantly related.

**Folate forms in relation to maternal MTHFR C677T and RFC-1 G80A genotypes**

Maternal concentrations of the primary folate forms according to maternal genotypes for MTHFR C677T and RFC-1 G80A are shown in Table 6. None of the folate-form concentrations differed between genotypes.

**DISCUSSION**

It has been a matter of debate whether the presence of unmetabolized folic acid in maternal or fetal circulation can have adverse effects in the developing fetus. The question of whether folic acid supplementation during pregnancy might cause the accumulation of unmetabolized vitamin in maternal or fetal circulation is a very important issue. We tested the primary folate forms in pregnant women with and without folic acid supplementation (400 μg folic acid/d). In addition, we tested folate forms in samples of umbilical cord serum at delivery. The important finding in this study is that detectable but small amounts of folic acid were observed in cord serum at birth. Concentrations of unmetabolized folic acid in maternal serum were comparable with those in pregnant women from Ireland (19). Our results confirm those of Sweeney et al (19), who showed detectable amounts of folic acid in the majority of maternal and cord blood samples from a population with voluntary folic acid fortification. As in the study by Sweeney et al (19), we observed a direct correlation between folate and unmetabolized folic acid concentrations in cord blood and in maternal serum. Concentrations of unmetabolized folic acid in cord blood in our study were not related to maternal serum folic acid and seemed not to be explained by vitamin use.

Detectable concentrations of unmetabolized folic acid in blood have been reported in studies in countries with fortification programs. However, folic acid has also been detected in subjects not taking any vitamins before fortification became mandatory in the United States (6). Fortification with folic acid is not mandatory in Germany. However, several breakfast cereals and juices are fortified with folic acid. This might explain why plasma concentrations of folic acid in pregnant women were not predicted by the use of a folic acid supplement during pregnancy and were probably related to the consumption of fortified foods. Both total folate and folic acid concentrations in non-supplemented women in this study were comparable with data from the Framingham Study for nonsupplemented subjects before fortification of grain products with folic acid (6). Folic acid concentrations were also detectable in cord blood from births in Ireland [mean: 0.23 μg/L (0.52 nmol/L); $n = 12$] and in newborns from mothers who did not receive supplemental folic acid (19). The authors supposed that this was related to the consumption of foods fortified with folic acid (19). We observed no correlation between maternal and fetal concentrations of folate forms. In contrast to concentrations of folic acid, concentrations of total folate and 5-MTHF and the proportion of 5-MTHF of total folate were higher in cord than in maternal serum (Table 5). This argues against the accumulation of folic acid by the fetus.
Nevertheless, because total folate was higher in women supplemented with folic acid (Table 2) than in nonvitamin users, we cannot exclude that the study might be underpowered for the detection of significant differences in cord blood folic acid according to maternal vitamin use. Further investigations for a larger sample size are necessary. Alternatively, because folate is an important antioxidant (25), and folic acid is the oxidized form of the vitamin, we speculate that folic acid might be produced in vivo from the internal nonenzymatic oxidation of 5-MTHF or THF.

Pfeiffer et al (26) reported that nonfasting blood samples containing folate concentrations >50 nmol/L were more likely to contain unmetabolized folic acid than were samples with lower concentrations. This is supported by our current results showing a significant positive correlation between folic acid and 5-MTHF or total folate concentrations, which further supports our view that folic acid might originate from the nonenzymatic oxidation of other forms of folate.

In a subset of the Framingham Offspring Study that included individuals who were exposed and other individuals who were not exposed to folic acid fortification, the 85th percentile of folic acid concentrations in plasma was 1.35 nmol/L (27). In our study, with only one outlier, the highest concentrations of folic acid measured in maternal and cord sera were 1.15 and 0.71 nmol/L, respectively. Our results are consistent with a lower intake of folic acid compared with intakes of folic acid in populations from countries where fortification is carried out. For example, concentrations of folic acid (mean: 0.25 nmol/L) and 5-MTHF (mean: 19.0 nmol/L) in nonsupplemented people from the Framingham Offspring Study before fortification were comparable with those in nonsupplemented women in our study (Table 2)(6). In addition, Kalmbach et al (6) showed that approximately three-quarters of persons who used supplements, which typically provided 400–800 µg folic acid, before the fortification era and persons who did not use supplements after fortification had detectable circulating folate concentrations. In our study, 38 of 87 pregnant women (44%) had detectable concentrations of folic acid, but this was not completely explained by supplemental folic acid (Table 4). Furthermore, pregnant women in our study who received 400 µg folic acid/d throughout their pregnancy had lower circulating folic acid concentrations than did supplemented participants from the Framingham Study (6), which might be related to physiologic factors such as enhanced folic acid reduction during pregnancy.

The low detection limit of our assay was also comparable with that of Kalmbach et al (6). Compared with our study, Sweeney et al (20) defined the LOD for a signal-to-noise ratio of 3, which would have yielded an LOD of 0.13 nmol/L if we considered the same definition. Other factors responsible for between-individual variations in the ability to use folic acid have been discussed. Recently, Bailey and Ayling (15) reported significant between-subject variations in the liver activity of dihydrofolate reductase (DHFR), which is the enzyme that reduces folic acid to 7,8-dihydrofolate and then to THF. In one study, the intake of >500 µg folic acid/d caused a higher incidence of individuals with high folic acid in the presence of a DHFR polymorphism that was supposed to decrease the capacity of the enzyme to reduce folic acid (27). However, in the study by Bailey et al (14), the DHFR activity measured in fresh liver extracts seemed to be a limiting factor only when folate intake exceeded the tolerable upper intake of 1 mg folate/d, which was probably not a relevant factor in our study. As suggested by Bailey et al (14), our findings in this nonfortified population imply that most of the folic acid in the region of 400 µg folic acid given to pregnant women was converted to active folates in most individuals.

Taken together, our results show that concentrations of 5-MTHF and THF, but not of folic acid, were higher in cord than in maternal serum. Maternal folic acid supplement use did not explain the detection of unmetabolized folic acid in maternal blood or cord blood. It seems that in the majority of pregnant women, folic acid supplied during pregnancy is not likely to accumulate in the fetus, in contrast to 5-MTHF and THF, which accumulate in the fetus.

The authors’ responsibilities were as follows—RO: conceived the original idea for the study, participated in the concept and design of the statistical analyses, and wrote the manuscript; MK: participated in analysis of folate forms and genotyping; SHK: established the method for folate-form analysis; WM: was responsible for recruiting pregnant women for the study and participated in study design, sample collection, and gathering of clinical data; and WH: participated in the study design and data interpretation and provided input for the final manuscript. None of the authors reported a conflict of interest.

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