Steady state folate concentrations achieved with 5 compared with 1.1 mg folic acid supplementation among women of childbearing age\(^1\)–\(^3\)

Patricia Nguyen, Carolyn Tam, Deborah L O’Connor, Bhushan Kapur, and Gideon Koren

ABSTRACT

Background: Synthetic folic acid (0.4–1.0 mg) consumed during the periconceptional period has been shown to reduce the risk of neural tube defects. Women with poor supplement adherence or a previous pregnancy affected by a neural tube defect may need to take higher doses of folic acid (4–5 mg). However, there are limited data on the pharmacokinetics of higher folic acid doses.

Objective: Our aim was to compare steady state folate concentrations in women of childbearing age who took 5 or 1.1 mg folic acid daily for 30 wk.

Design: Forty nonpregnant women aged between 18 and 45 y, who did not take folic acid supplements, were enrolled in the study. Subjects were randomly assigned to take either 5 or 1.1 mg folic acid daily for 30 wk. Plasma and red blood cell (RBC) folate concentrations were measured at baseline and at weeks 2, 4, 6, 12, and 30.

Results: There was no significant difference in baseline RBC folate concentrations between the 2 groups (1121 ± 410 and 1035 ± 273 nmol/L, for the 5- and 1.1-mg folic acid groups, respectively). Significant differences in RBC folate were detected between groups at weeks 4, 6, 12, and 30. RBC folate concentrations by week 30 were 2339 ± 782 and 1625 ± 339 nmol/L for the 5- and 1.1-mg folic acid groups, respectively.

Conclusion: The use of 5 mg folic acid among women of childbearing age produced higher blood folate concentrations, with a faster rate of folate accumulation, compared with 1.1 mg folic acid. *Am J Clin Nutr* 2009;89:844–52.

INTRODUCTION

In 1995 Daly et al (1) determined that the risk for a pregnancy affected by neural tube defects (NTDs) increased when maternal plasma folate and red blood cell (RBC) folate concentrations were <15.9 and 906 nmol/L, respectively. This is the only published study that has investigated the dose-response relation between maternal folate status and risk for NTDs; however, it demonstrates that an inverse relation does exist. Although food fortification with folic acid was implemented in North America in 1998 and folic acid supplements are available, achieving protective folate concentrations (ie, ≥906 nmol/L) remains a challenge among some women of childbearing age. A recent study revealed that 40% of childbearing-age women and 36% of pregnant women in Ontario, Canada, had RBC folate concentrations <906 nmol/L (2).

Possible explanations include low consumption of folate-rich or fortified foods, elevated requirements for folate (ie, folate malabsorption, genetic polymorphisms for enzymes in folate metabolism), and poor adherence to folic acid supplements. We published a study on pregnant women taking prenatal multivitamins, and our results indicated that adherence rates ranged from 0% to 100%, with a mean of only 55% even under optimal conditions of supervision and motivation (3). It has been reported that use of folic acid supplements among women of childbearing age during the periconceptional period is 40–60% (4–7). However, other investigators have reported the average use of folic acid before pregnancy to be as low as 23% (8–10).

Currently, it is recommended that women of childbearing age consume a minimum of 0.4 mg folic acid daily (from fortified foods and/or supplements), and most over-the-counter prenatal multivitamins contain 1 mg folic acid. The standard of care for women with a family history of NTD pregnancy or who take anticonvulsant medication is to take 5 mg folic acid daily before and during pregnancy (11, 12). The periconceptional use of 5 mg folic acid outside this specific group of women is uncommon. The Society of Obstetricians and Gynecologists of Canada now recommends 5 mg folic acid under a broader list of indications, including poor adherence (13). Wald et al (14) proposed that supplementation with 5 mg folic acid is needed to render maximum protection against NTDs (ie, a 90% risk reduction) among women of childbearing age, particularly when food fortification has not been implemented.

There are limited data on the pharmacokinetics of higher doses of folic acid. Before 5 mg folic acid can be recommended for greater use among women of childbearing age, it is important to...
examine its pharmacokinetics. The objective of the present study was to compare steady state blood folate concentrations in healthy women of childbearing age who were randomly assigned to take either 5 or 1.1 mg folic acid daily for 30 wk.

SUBJECTS AND METHODS

Recruitment of study participants

We recruited female volunteers through the Motherisk Program (The Hospital for Sick Children, Toronto, ON, Canada) beginning in March 2007. Motherisk is a counseling program that provides information to women on the safety or risk to a developing fetus and newborn of maternal exposure to drugs, chemicals, radiation, and disease. We included healthy nonpregnant women aged between 18 and 45 y, with normal renal function, who had not been taking multivitamins or folic acid supplements for ≥6 mo before their study participation. We excluded women with chronic medical conditions (ie, hypertension, diabetes, epilepsy, depression, thyroid problem) and those taking medication recurrently, including oral contraceptives, anticonvulsants, or other folate antagonists such as methotrexate. Other exclusion criteria were hypersensitivities to any of the ingredients in the study multivitamins and a family history or a previous pregnancy affected by an NTD. Subjects provided written informed consent, and the study was approved by the Ethics Review Board at the Hospital for Sick Children.

Study design

Our study was designed as a prospective, randomized, 2-arm, open-label, interventional study. After study enrollment, the pharmacy department at the Hospital for Sick Children assigned subjects randomly to receive prenatal multivitamins containing either 1.1 or 5 mg folic acid. The study coordinator and subjects were not blinded because it was not feasible to modify the appearance of tablets or product packaging. Women randomly assigned to the 1.1-mg folic acid group were instructed to take a daily prenatal multivitamin containing 1.1 mg folic acid (PregVit; Duchesnay Inc, Laval, PQ, Canada) for 30 wk, whereas women in the 5-mg folic acid group were instructed to take a daily prenatal multivitamin containing 5 mg folic acid (PregVit-Folic 5; Duchesnay Inc) for 30 wk. Both prenatal multivitamins were identical in the content of other vitamins and minerals, except folic acid, and were the same tablet size. Both prenatal multivitamins were prescribed as 2 tablets/d, with 1 tablet (pink) ingested at any time in the morning and 1 tablet (blue) ingested at any time in the evening. The morning and evening tablets contained different vitamins and minerals (Table 1); therefore, subjects did not receive a double dosing of micronutrients. Only the evening tablet contained folic acid.

Baseline measurements were collected from all subjects and included taking a 5-mL blood sample after at least a 6-h fast to measure initial plasma and RBC folate concentrations. Each subject completed a validated food-frequency questionnaire to document dietary folate intake during the 6 mo before study participation (15). Subjects were provided with an 8-wk supply of their randomly assigned prenatal multivitamins, and the supply was renewed at weeks 6 and 12. If tablets were missed, subjects were instructed to leave the tablet in the blister pack-aging and continue with the next tablet. Subjects were also instructed not to discard unused tablets or multivitamin packaging and to return them to the study coordinator for pill count. Subjects returned to the hospital at weeks 2, 4, 6, 12, and 30 (±3 d) to provide fasted blood samples (5 mL each) for folate measurements. At the final appointment (week 30), the food-frequency questionnaire was completed to document dietary folate intake during the 30 wk (~6–7 mo) of study participation.

Measured outcomes

First, the primary endpoints were plasma and RBC folate concentrations measured at baseline and at weeks 2, 4, 6, 12, and 30 by using a microbiological assay (16). Second, unmetabolized folic acid concentrations were measured in plasma at baseline and at week 30 by using HPLC. Third, we monitored plasma vitamin B-12 concentrations at baseline and at week 30 by using a solid-phase competitive chemiluminescent enzyme immunoassay. The Block Dietary Folate Equivalents (DFE) screener (Block Dietary Data Systems, Berkeley, CA) was used to document dietary folate intake. The Block DFE screener is a validated food-frequency questionnaire that quantifies folate intake in adults during the previous 6 mo (15). All questionnaires were processed by the Block Dietary Data Systems, and the data were organized into four categories of daily folate intake (in μg): 1) folate from natural-folate foods; 2) folate from fortified foods; 3) total folate, which was the sum of folate from natural-folate and fortified foods; and 4) total DFE in which the quantity of synthetic folic acid in the diet was multiplied by 1.7 to account for its greater bioavailability compared with natural sources of folate (17). Finally, pill count was used to monitor adherence to supplement regimens. Pill intake was defined as the number of pills ingested as a percentage of the total number of pills prescribed.

Blood sample preparation

Fasting blood samples (5 mL) were collected in 2 evacuated tubes containing EDTA. The tubes of blood were quickly shielded

| TABLE 1 Composition of PregVit and PregVit-folic 5 |
|-------------------------|-------------------------|
| **Morning tablet** | **Evening tablet** |
| β-Carotene (2700 IU) | Folic acid |
| Vitamin E (30 IU) | 1.1 mg (if prescribed PregVit) |
| Vitamin C (120 mg) | 5 mg (if prescribed PregVit-folic 5) |
| Thiamine (3 mg) | Vitamin B-12 (12 μg) |
| Riboflavin (3.4 mg) | Vitamin D (250 IU) |
| Niacinamide (20 mg) | Calcium (300 mg) |
| Vitamin B-6 (10 mg) | |
| Pantothenic acid (5 mg) | |
| Magnesium (50 mg) | |
| Iodine (0.15 mg) | |
| Iron as ferrous fumarate (35 mg) | |
| Copper (2 mg) | |
| Zinc (15 mg) | |

PregVit, prenatal multivitamin containing 1.1 mg folic acid; PregVit-folic 5, prenatal multivitamin containing 5 mg folic acid (both manufactured by Duchesnay Inc, Laval, PQ, Canada).
from light and placed on ice; sample preparation occurred within 2 h of blood collection. Before sample preparation, 2 capillary tubes were used to collect blood for hematocrit readings.

For the whole-blood preparation, a 1% (wt:vol) solution of ascorbic acid was made with deionized water. We transferred 100 μL of whole blood into each of 3 labeled cryovial tubes, added 900 μL of 1% ascorbic acid solution to each, and vortexed briefly to mix. Ascorbic acid serves as an antioxidant and assists in the preservation of (RBC) folate. Whole-blood preparations were incubated at 37°C to convert folates to their microbiologically assayable form, after which they were immediately stored at −80°C.

The remaining blood in the evacuated tubes was centrifuged at 1500 g at 4°C for 20 min to separate the plasma from the RBCs. Once the plasma was collected, we transferred 500 μL of plasma into each labeled cryovial tube to make a duplicate set available for vitamin B-12 analysis. Sodium ascorbate was added to the remaining plasma to make a 1% (wt:vol) solution, mixed, and stored in aliquots at −80°C.

**Microbiological assay**

Plasma and whole-blood folate were measured by microbiological assay, as described by Molloy and Scott (16) with modifications, by using the test organism *Lactobacillus rhamnosus* (ATCC 7469; American Type Tissue Culture Collection, Manassas, VA). *L. rhamnosus* responds to both folic acid and its reduced, metabolically active derivatives. Accuracy and interassay variability were assessed by using a whole-blood standard with a certified value of 29.5 nmol/L (whole blood 95/528; National Institute of Biological Standards and Control, Hertfordshire, United Kingdom). Our analysis yielded a folate content of 30.6 ± 1.0 nmol/L with an interassay CV of 3.4%.

**HPLC analysis of unmetabolized folic acid in plasma**

Folates were purified from enzyme-treated plasma samples by affinity chromatography with the use of immobilized bovine milk FBP isolated from dried whey milk powder. A detailed description of the methods and materials used to prepare and store affinity columns is published elsewhere (18). The recovery from the affinity column, as measured with tritiated folic acid (Amersham Pharmacia Biotech, Piscataway, NJ), was 85.5% ± 6% (mean ± SD). The total folate binding capacity exceeded 500 μmol/L solid phase. The folic acid from purified plasma samples was identified by using ion-pair HPLC with electrochemical detection as described in detail by Bagley and Selhub (19, 20) and by Belz and Nau (21). The HPLC system consisted of a P580 pump with an ASI-100 autosampler, a 250 × 4.6-mm Betalis Phenyl analytic column, and an ED50 electrochemical detector with set-up shift and Ag/AgCl reference potential, which was managed by Chromelon version 6.2 software. All parts were purchased from Dionex (Oakville, ON, Canada), except for the phenyl analytic column (Keystone Scientific, Thermo Electron Corporation, Waltham, MA). The mobile phase was delivered at a flow rate of 0.75 mL/min and maintained at 25°C (112 mmol/L potassium phosphate, 240 mmol/L phosphoric acid), 7% B (800 mL/L acetonitrile), and 68% water for the first 10 min. From 10 to 40 min, the concentration of B was raised linearly to 20%, which provided the gradient. The folic acid derivative was identified on the basis of the retention time (22.3 min) compared with the electrochemical response of the peak of the folic acid standard (Sigma, Oakville, ON, Canada). The between-run precision (1.4%) was determined by analyzing aliquots of a plasma control.

**Statistical analysis**

A sample size of 40 subjects (20 subjects/group) was calculated to be sufficient to assess the differences in steady state blood folate concentrations between the 2 groups with a power of 85% and α of 5%. The sample size calculations factored in the ~30% difference in risk reduction for NTDs between the 2 folic acid doses, as suggested by Wald et al (14). The primary analysis was to compare the plasma and RBC folate concentrations between the 2 groups by using SAS for WINDOWS (version 9.1; SAS Institute Inc, Cary, NC), and the results were considered significant at *P* < 0.05. All data were tested for normal distribution (PROC UNIVARIATE procedure), and skewed data were transformed for statistical analyses. Blood folate data were analyzed by using repeated measures (SAS PROC MIXED) with dose (5 or 1.1 mg folic acid) and time (baseline, week 2, week 4, week 6, week 12, and week 30) treated as main effects and a dose-by-time interaction. Tukey’s comparison procedure was used for pairwise comparison if a statistically significant dose-by-time interaction was found. Using the same statistical approach, we also compared the following: 1) dietary folate intake, 2) plasma vitamin B-12 concentrations, and 3) unmetabolized folic acid concentrations at baseline and at week 30.

**Secondary analysis**

We modeled the impact of adherence and folic acid dose on population folate status. We acquired baseline RBC folate concentrations for a large sample (*n* = 1494) of healthy nonpregnant women of childbearing age in Ontario, Canada, in which 41% had RBC folate concentrations <906 nmol/L (2). Using the RBC folate concentrations measured for the 40 subjects in the present study, we determined the change (Δ) between concentrations at baseline and at week 12. On the basis of the RBC-folate-concentration time curves, week 12 was the earliest time point at which steady state concentrations were measured. For each subject, we calculated the ratio of Δ to pill intake (Δ/Pill intake), and then we determined the geometric mean ratio for each treatment group. Using arbitrarily selected rates of adherence (ie, pill intake of 100%, 80%, and 20%), we calculated the Δ that could be achieved with either 5 or 1.1 mg folic acid. The calculated Δs were then added to the baseline RBC folate concentrations of the large sample of Ontario women (2). We then determined the proportion of women with concentrations still <906 nmol/L at each adherence rate and for each folic acid dose.

We also modeled the impact of folate accumulation and folic acid dose on population folate status by using the RBC folate concentrations for the same sample (*n* = 1494) of Ontario women. On the basis of the concentration-time curves for the 40 subjects in the present study, the accumulation rate was determined by the slope of the curve and defined as the change in RBC folate concentration per week (nmol/L · wk⁻¹). The mean accumulation rate was determined for each treatment group, and we calculated the change in RBC folate concentrations that
could be achieved at weekly increments for the 2 doses of folic acid. The calculated changes were then added to the baseline RBC folate concentrations reported for the large sample of Ontario women (2). Once again, we determined the proportion of women with concentrations still <906 nmol/L at each weekly increment and for each folic acid dose.

For both secondary analyses, there is a caveat that the baseline RBC folate concentrations measured for the sample (n = 1494) of Ontario women were determined by various laboratory techniques and at different laboratory sites. We are aware that there are differences between techniques in accurately measuring blood folate concentrations (22).

RESULTS

Between March 2007 and February 2008, we enrolled 40 healthy nonpregnant women of childbearing age for the present study (Figure 1, Table 2). Other women were excluded because they were taking oral contraceptives, because they could not commit to 30 wk of supplementation, or because they did not feel comfortable with blood work (Figure 1). Of the 40 enrolled subjects, 20 were randomly assigned to the 5-mg folic acid group and 20 were randomly assigned to the 1.1-mg folic acid group; however, 1 subject from each group dropped out of the study due to anxiety with blood work and difficulty committing to the study protocol. The remaining 19 subjects in each group completed the study. We included all data collected from the 2 subjects who dropped out. Data included baseline measurements for both subjects and all measurements at week 2 for one subject in the 5-mg folic acid group.

There was no significant difference between the 2 groups in daily dietary folate intake (Table 3). Dietary folate intake remained consistent from the time at which subjects started the study (baseline) to when they completed the study (week 30). Therefore, any substantial changes in blood folate concentrations were most likely due to the study intervention.

No significant difference was detected in baseline plasma folate concentrations between the 5- and 1.1-mg folic acid groups [48.8 ± 14.5 and 49.4 ± 15.7 nmol/L, respectively (P = 0.96)]. However, there were significant differences between groups at weeks 2, 4, 6, 12, and 30 (Figure 2). Plasma folate concentrations increased to 165.3 ± 109.9 nmol/L for the 5-mg folic acid group and to 96.8 ± 41.1 nmol/L for the 1.1-mg folic acid group by week 30. No significant difference was detected in baseline RBC folate concentrations between the 2 groups, which were measured as 1121 ± 410 nmol/L for the 5-mg folic acid group and as 1035 ± 273 nmol/L for the 1.1-mg folic acid group (P = 0.51). However, there were significant differences between groups at week 4 (P = 0.006), week 6 (P = 0.001), week 12 (P = 0.0002), and week 30 (P = 0.0005) (Figure 3). Among the 40 subjects, 13 (33%) had baseline RBC folate concentrations <906 nmol/L. By week 30, RBC folate concentrations increased to 2339 ± 782 and to 1625 ± 339 nmol/L for the 5- and 1.1-mg folic acid groups, respectively.

FIGURE 1. Study sample (n = 40) recruitment flow chart.
Table 2
Characteristics of subjects1

<table>
<thead>
<tr>
<th></th>
<th>5-mg folic acid group (n = 20)</th>
<th>1.1-mg folic acid group (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>35 ± 7 2</td>
<td>33 ± 6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63.6 ± 19.3</td>
<td>63.4 ± 10.7</td>
</tr>
<tr>
<td>Gravidity (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never been pregnant</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Previously pregnant</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Maternal race (n)</td>
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<tr>
<td>White</td>
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<tr>
<td>Hispanic</td>
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<tr>
<td>South Asian</td>
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<td>1</td>
</tr>
<tr>
<td>Southeast Asian</td>
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<td>4</td>
</tr>
<tr>
<td>Education</td>
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<td>Homemaker —</td>
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<td>Part-time employment</td>
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<td>Full-time employment</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Student</td>
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<td>2</td>
</tr>
<tr>
<td>Reported smoking nicotine</td>
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<td>1</td>
</tr>
<tr>
<td>Substance exposure (n)</td>
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<td></td>
</tr>
<tr>
<td>Reported occasional/social</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>alcohol consumption</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

1 There were no significant differences between groups, as determined by Student’s t test and chi-square test.
2 Mean ± SD (all such values).
3 Subjects earned a postsecondary education at the college or university level.
4 Subjects were not taking medication(s) for chronic indications during the study. Two subjects reported rare use of ibuprofen for headaches, one subject reported rare use of topical minocyn for mild acne.

At baseline, unmetabolized folic acid was detected in 70% and 65% of subjects in the 5- and 1.1-mg folic acid groups, respectively. By week 30, unmetabolized folic acid was detected in 58% of both groups (Table 4). There was no significant difference in the concentrations of unmetabolized folic acid between the 2 groups, and detected concentrations were much lower relative to total plasma folate at baseline and at week 30 (Table 4). There was also no significant correlation between unmetabolized folic acid concentrations and adherence rates (df = 37, r = 0.093, P > 0.5).

Baseline vitamin B-12 concentrations were 388 ± 148 pmol/L for the 5-mg folic acid group and 389 ± 129 pmol/L for the 1.1-mg folic acid group. By week 30, vitamin B-12 concentrations increased to 555 ± 231 pmol/L for the 5-mg folic acid group and to 547 ± 292 pmol/L for the 1.1-mg folic acid group. There was no significant difference between groups. Vitamin B-12 concentrations increased from baseline to week 30 for both groups, which was most likely due to the fact that the prenatal multivitamins used in the study contained 12 μg of vitamin B-12.

There were 5 reports of nausea, 3 of constipation, and 3 of abdominal pain or discomfort; there were also single reports of swallowing difficulty, heartburn, and diarrhea. However, there were no significant differences in adverse events between groups. The reported adverse events did not appear to affect the adherence of either group, because the mean pill intake was 86 ± 17% for the 5-mg folic acid group (range: 38–99.5%) and 82 ± 21% for the 1.1-mg folic acid group (range: 30–100%).

Modeling changes in RBC folate concentrations with respect to adherence

On the basis of the change (Δ) in RBC folate concentrations measured at baseline and at week 12, we determined the mean Δ to be 1152 ± 515 nmol/L for the 5-mg folic acid group and 488 ± 276 nmol/L for the 1.1-mg folic acid group. A mean ratio of Δ to pill intake was calculated for the 5- and 1.1-mg folic acid groups as 11.8 and 4.4 nmol/L/% pill intake, respectively. We applied the mean ratios to a large sample (n = 1494) of healthy nonpregnant women of childbearing age in Ontario, in which 41% had RBC folate concentrations <906 nmol/L (2). When adherence is ideal at 100% pill intake, either 5 or 1.1 mg folic acid could substantially improve RBC folate concentrations such that the 41% is reduced to 0–1% (Table 5). However, at a poor adherence of only 20% pill intake, 5 mg folic acid could still render greater improvements in RBC folate (ie, 41% reduced to 11%) compared with 1.1 mg folic acid (ie, 41% reduced to 27%; Table 5).

Modeling changes in RBC folate concentrations with respect to accumulation rate

On the basis of the slopes of the concentration time curves of 5- compared with 1.1-mg folic acid supplementation (Figure 3), the mean accumulation rates (change in RBC folate per week) for the 5- and 1.1-mg folic acid groups were 96.8 and 41.4 nmol/ L/wk, respectively. We applied the mean accumulation rates to the same data set of Ontario women in which 41% had RBC folate concentrations <906 nmol/L (2). We calculated that, after 1 wk of supplementation with 5 mg folic acid, the proportion of women with RBC folate <906 nmol/L could be reduced to 25% (Table 6). By week 6, we calculated that all of the women in the sample could have RBC folate concentrations >906 nmol/L (ie, 41% reduced to 0%) when taking 5 mg folic acid. If the women were taking 1.1 mg folic acid, 10% might require additional weeks of supplementation before the targeted folate concentration was achieved (Table 6). However, this model assumes ideal adherence (ie, daily pill intake).
DISCUSSION

To the best of our knowledge, the present study is the first to investigate steady state blood folate concentrations achieved with daily 5-mg folic acid supplementation in women of childbearing age. We recruited female volunteers of relatively high socioeconomic status (SES); thus we expected most, if not all, of the women to have RBC folate concentrations thought to be protective against NTDs (ie, ≥906 nmol/L). Thirty-three percent of subjects had RBC folate concentrations <906 nmol/L, which was consistent with other studies that examined folate status in women of childbearing age after fortification was implemented (2, 23). Considering that 10–30% of women with moderate-to-high SES have RBC folate concentrations <906 nmol/L, it is conceivable that many more women with low-to-moderate SES would have RBC folate concentrations below this cutoff (24, 25). One explanation for this may be low consumption of folate-rich and fortified foods. Sixty-five percent of our subjects consumed <400 µg of total food folate per day, even though a minimum intake of 400 µg folic acid daily is recommended to reduce the risk of NTDs (26). No change was evident when dietary folate was assessed by week 30. These findings are consistent with other studies that have also documented low intake of folate-rich and fortified foods among women of childbearing age (27–29).

A common challenge with taking folic acid supplements is adherence. We expected good adherence from the 40 subjects, particularly because we supplied the prenatal multivitamins and participation was under ideal conditions of supervision and motivation. Thus, 70% of subjects demonstrated at least 80% pill intake. In contrast, in a previous study we determined that adherence among pregnant women taking prenatal multivitamins under ideal conditions was suboptimal with a mean adherence rate of 50% that ranged widely from 0% to 100% (3). Use of folic acid supplements during the periconceptional period has also been reported to be suboptimal (ie, 40–60% and as low as 23%) (6–9, 30–32).
TABLE 4
Unmetabolized folic acid concentrations of fasted nonpregnant women of childbearing age

<table>
<thead>
<tr>
<th></th>
<th>5-mg folic acid group</th>
<th>1.1-mg folic acid group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (n = 20)</td>
<td>Week 30 (n = 19)</td>
</tr>
<tr>
<td>Total plasma folate</td>
<td>48.8 ± 14.5 (2)</td>
<td>49.4 ± 15.7</td>
</tr>
<tr>
<td>Proportion with</td>
<td>70</td>
<td>65</td>
</tr>
<tr>
<td>detected</td>
<td></td>
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</tr>
<tr>
<td>Plasma concentration of detected unmetabolized folic acid (nmol/L)</td>
<td>8.6 ± 6.4</td>
<td>11.2 ± 10.5</td>
</tr>
</tbody>
</table>

1 Mean ± SD (all such values).
2 There was no significant difference between groups, P > 0.05 (Tukey’s pairwise comparison).

There is limited information on the pharmacokinetics of folic acid at high doses (ie, 5 mg). We recently published a study comparing the single-dose pharmacokinetics of 5 compared with 1.1 mg folic acid in nonpregnant women of childbearing age (33). We determined that single-dose ingestion of folic acid follows linear pharmacokinetics such that a 5-fold increase in folic acid dose (ie, 1.1–5 mg) produced an ≈5-fold increase in pharmacokinetic measurements (ie, area under the curve, Cmax). However, our present study of women taking 5 or 1.1 mg folic acid for 30 wk suggests that repeated use of folic acid follows nonlinear pharmacokinetics because a 5-fold difference in folic acid dose produced only an ≈2-fold difference in steady state RBC folate concentrations. The nonlinear pharmacokinetics suggests that folate uptake at higher doses may involve a limiting mechanism such as passive diffusion or low dihydrofolate reductase activity (26, 34, 35).

TABLE 5
Estimated proportion of women with red blood cell (RBC) folate concentrations <906 nmol/L on the basis of theoretical supplementation with 2 different doses of folic acid at various adherence rates

<table>
<thead>
<tr>
<th>Initial proportion with RBC folate &lt;906 nmol/L</th>
<th>Adherence rates (% pill intake)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Folic acid</td>
<td></td>
</tr>
<tr>
<td>5 mg2</td>
<td>41</td>
</tr>
<tr>
<td>1.1 mg2</td>
<td>41</td>
</tr>
</tbody>
</table>

1 This is from a sample of 1494 healthy nonpregnant women of childbearing age. Of this sample of women, 41% initially had RBC folate concentrations <906 nmol/L (2). We assumed 100% pill intake (ie, supplement taken daily).
2 The accumulation rate was a 96.8-nmol/L change in RBC folate per week of supplementation on the basis of the steady state kinetics of 5-mg folic acid supplementation among 20 healthy nonpregnant women.
3 The accumulation rate was a 41.4-nmol/L change in RBC folate per week of supplementation on the basis of the steady state kinetics of 1.1-mg folic acid supplementation among 20 healthy nonpregnant women.

In contrast, Pietrzik et al (36) suggested a kinetics model for steady state RBC folate based on first-order kinetics, which is characteristic of linear pharmacokinetics. However, Pietrzik et al developed the model based on using 0.4 and 0.8 mg folic acid, whereas our present study examined the pharmacokinetics of higher doses of folic acid (ie, 1.1 and 5 mg). Further investigation is needed to confirm whether the pharmacokinetics of folic acid is specifically linear at lower doses and nonlinear at higher doses.

Our results were consistent with that of a study conducted in China in which women of childbearing age (n = 1108) were randomly assigned to folic acid interventions that lasted for 6 mo and included the following dosing regimens: 1) 4 mg daily, 2) 4 mg once per week, 3) 0.1 mg daily, and 4) 0.4 mg daily (37). Baseline RBC folate concentrations for all groups were <906 nmol/L. Results from this study indicated that women who took 4 mg folic acid daily increased their RBC folate concentrations at a faster rate compared with women in the other treatment groups, which was also observed in our findings of women taking 5 mg folic acid daily for 30 wk (∼7 mo).

With our population modeling, we demonstrated the impact of supplement adherence on folate status. The implication from our modeling is that women with RBC folate concentrations <906 nmol/L and poor adherence can benefit from taking a higher dose of folic acid (ie, 5 mg) during the periconceptional period. We also demonstrated the impact of the folate accumulation rate. The implication is that women with RBC folate concentrations <906 nmol/L and a short periconceptional period (ie, unplanned pregnancy) can rapidly achieve the targeted folate concentration by taking a higher dose of folic acid. It is relevant to note that neural tube closure is complete by 28 d (∼4 wk) of human development. As described by Bar-Oz et al (2), 5 mg acts as a booster dose to efficiently elevate blood folate concentrations, especially when needed amid poor adherence and/or time limitations.

Folic acid toxicity is generally not a concern because it is a water-soluble B vitamin and can undergo renal elimination. It is considered nontoxic across a range of recommended doses (0.4–5 mg/d) for healthy individuals. Although there is concern that high folate concentrations will mask vitamin B-12 deficiency, one
study suggested that macrocytosis, an indicator of vitamin B-12 deficiency, can still be detected amid high serum folate concentrations (38). New legislation in Canada also requires that products with a natural product number, containing ≥200 µg folic acid, either must supplement with vitamin B-12 according to the Recommended Dietary Allowance or advise consumers to consult a health care practitioner regarding vitamin B-12 intake (39).

Several published studies have reported that there is an association between high folic acid intake and increased risk for colorectal cancer (40–42). Although this issue requires further confirmation, it is important to emphasize that the use of 5 mg folic acid among women of childbearing age is intended for a defined period of time (ie, periconceptional period) under specific circumstances (ie, poor adherence, family history of NTDs).

Investigators have also expressed concern over the systemic presence of unmetabolized folic acid, including its effect on natural killer cells (34, 43, 44). We determined that the prevalence and concentrations of detected unmetabolized folic acid in women taking 5 mg folic acid were low and not significantly different compared with women taking 1.1 mg folic acid. Although this was unexpected, we examined blood samples from subjects who had fasted, and other investigators have also found that plasma concentrations of unmetabolized folic acid were minimal, with folic acid consumption ranging from 0.1 to 4 mg (34, 45, 46). Unmetabolized folic acid in blood from fasting subjects tends to be lower compared with postprandial blood; one study determined that unmetabolized folic acid concentrations peaked 80 min after ingestion of 3 mg folic acid but declined to baseline afterward (47).

In summary, our data showed that the use of 5 mg folic acid among women of childbearing age produced higher blood folate concentrations, with a faster rate of folate accumulation, than 1.1 mg folic acid. Therefore, health care practitioners can consider recommending 5 mg folic acid to women of childbearing age who may be at risk for suboptimal folate status (ie, <906 nmol/L RBC folate) for the prevention of NTDs after the proper assessment of diet, adherence capabilities, and other criteria for elevated folate requirements. This approach of individualizing folic acid counseling can optimize the practice of folic acid supplementation.

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