Folate Status Response to Controlled Folate Intake in Pregnant Women\textsuperscript{1–4}

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ABSTRACT A metabolic study (84-d) was conducted to investigate the folate status response of pregnant subjects ($n = 12$) during their second trimester and nonpregnant controls ($n = 12$) to folate intakes approximating the current (400 $\mu g/d$) and former (800 $\mu g/d$) recommended dietary allowance (RDA). The overall goal of the study was to provide metabolic data to assist in the interpretation of the current RDA for folate. Subjects were fed a controlled diet containing 120 $\pm$ 15 $\mu g/d$ (mean $\pm$ sd) folate and either 330 or 730 $\mu g/d$ synthetic folic acid. Outcome variables between and within supplementation groups were compared at steady state. Serum folate was higher ($P < 0.05$) in pregnant women consuming 850 compared with 450 $\mu g/d$ (44.6 $\pm$ 13.4, 26.3 $\pm$ 11.3 nmol/L, respectively, mean $\pm$ sd). No differences ($P > 0.05$) were detected in serum folate between pregnant and nonpregnant women within the same supplementation group. Urinary 5-methyl-tetrahydrofolate excretion was greater ($P < 0.05$) in pregnant women consuming 850 compared with 450 $\mu g/d$ (198.0 $\pm$ 100.4, 9.5 $\pm$ 3.2 nmol/L, respectively). No differences ($P > 0.05$) in 5-methyl-tetrahydrofolate excretion were detected between pregnant and nonpregnant women within supplementation groups. Differences ($P < 0.05$) were not detected in red cell folate between pregnant women consuming either 450 or 850 $\mu g/d$ (1452.5 $\pm$ 251.8, 1733.5 $\pm$ 208.5 nmol/L, respectively) or between pregnant and nonpregnant women consuming 450 $\mu g/d$. Our data suggest that 450 $\mu g/d$ (dietary folate + synthetic folic acid) is sufficient to maintain folate status in pregnant women. This level of intake equates to $\sim$600 $\mu g/d$ dietary equivalents, assuming 50 and 75% availability of dietary folate and synthetic folic acid consumed with meals, respectively. J. Nutr. 127: 2363–2370, 1997.

KEY WORDS: \textbullet folate \textbullet requirements \textbullet humans \textbullet pregnant

Folate plays a major coenzymatic role in one-carbon metabolism and is a key participant in the biosynthesis of DNA, RNA and certain amino acids (Wagner 1995). The body’s requirement for folate is thus related to the amount of cellular reproduction occurring at any particular time (Hibbard 1964). Pregnancy is associated with an enormous increase in cellular proliferation as a result of uterine enlargement, expansion of blood volume, placental development and fetal growth (Cunningham et al. 1993).

The recommended dietary allowance (RDA)\textsuperscript{5} for folate was reduced by approximately one half in the 10th edition from and underreporting of dietary intake. Controlled metabolic studies addressing the issue of folate requirements in nonpregnant women (O’Keefe et al. 1995, Sauberlich et al. 1987) clearly illustrated that 180 $\mu g/d$ was not sufficient to maintain normal folate status and suggested that 300–400 $\mu g/d$ more adequately met the definition of an RDA. The potential increase in folate requirements associated with rapid tissue growth during pregnancy is widely accepted; however, the increment above that required by nonpregnant women has not been ascertained.

The reduction in the folate RDA for nonpregnant women was the observation that folate intake estimated by population surveys including the Second National Health and Nutrition Examination Survey (NHANES II) (Life Sciences Research Office 1984) was $\sim$50% lower than the RDA reported in the 9th edition, and evidence of widespread folate inadequacy was lacking (Senti and Pilch 1984). Potential limitations to this approach have been reported (Bailey 1992 and 1995) and include weaknesses in analytical methodologies used to establish food composition tables (Gregory et al. 1990) and underreporting of dietary intake. Controlled metabolic studies addressing the issue of folate requirements in nonpregnant women (O’Keefe et al. 1995, Sauberlich et al. 1987) clearly illustrated that 180 $\mu g/d$ was not sufficient to maintain normal folate status and suggested that 300–400 $\mu g/d$ more adequately met the definition of an RDA. The potential increase in folate requirements associated with rapid tissue growth during pregnancy is widely accepted; however, the increment above that required by nonpregnant women has not been ascertained.

One rationale for reducing the RDA for nonpregnant women was the observation that folate intake estimated by population surveys including the Second National Health and Nutrition Examination Survey (NHANES II) (Life Sciences Research Office 1984) was $\sim$50% lower than the RDA reported in the 9th edition, and evidence of widespread folate inadequacy was lacking (Senti and Pilch 1984). Potential limitations to this approach have been reported (Bailey 1992 and 1995) and include weaknesses in analytical methodologies used to establish food composition tables (Gregory et al. 1990) and underreporting of dietary intake. Controlled metabolic studies addressing the issue of folate requirements in nonpregnant women (O’Keefe et al. 1995, Sauberlich et al. 1987) clearly illustrated that 180 $\mu g/d$ was not sufficient to maintain normal folate status and suggested that 300–400 $\mu g/d$ more adequately met the definition of an RDA. The potential increase in folate requirements associated with rapid tissue growth during pregnancy is widely accepted; however, the increment above that required by nonpregnant women has not been ascertained.

The reduction in the folate RDA for pregnant women was based largely on the findings of two studies (NRC1989). Chernar et al. (1968b) reported that 100 $\mu g/d$ synthetic folic acid, in addition to dietary folate, maintained red cell folate (RCF) concentration in pregnant women throughout gestation. Although dietary folate intake was estimated as 676 $\mu g/d$
after analyzing 111 24-h duplicate meals from 16 subjects (Chanarin et al. 1968a), the 10th edition RDA committee used an estimated dietary folate intake of 190 μg/d, which was subsequently reported by Bates et al. (1982) for this population. This estimate (Bates et al. 1982) in conjunction with Chanarin’s report that 100 μg/d plus diet maintained RCF was cited by the 10th RDA committee in support of 400 μg/d RDA (NRC 1989). Colman et al. (1975) found that maize fortified with 300 μg folic acid consumed with a constant diet maintained normal serum and RCF concentrations during the last 30 d of pregnancy in a nutritionally compromised, rural, African population. Interpretation of these studies is complex, and illustrates the importance of investigating folate requirements during pregnancy under controlled metabolic conditions.

McPartlin et al. (1993) estimated folate requirements in pregnant women at three different stages of gestation by quantifying the urinary excretion of the folate catabolites, p-amino-benzoylglutamate (pABG) and its acetylated derivative, acetamidobenzoylglutamate (apABG) in 24-h urine collections. This approach is based on the assumption that folate catabolites represent folate turnover and thus requirements. Pregnant women in their second trimester excreted twice as much apABG as pregnant women in their first trimester or nonpregnant controls. Folate requirement estimates were made by converting the quantities of pABG and apABG to “folate equivalents” on the basis of their molecular weights. McPartlin et al. (1993) estimated an RDA of 660 μg/d during the second trimester following adjustment for bioavailability and population variance.

The decline in folate status (both serum and red cell) during pregnancy has been well documented in both developed (Baley et al. 1980, Ek and Magnus 1981, Lowenstein et al. 1966, Qvist et al. 1986) and underdeveloped countries (Colman et al. 1975). Although overt megaloblastic anemia is infrequent in the United States, it seems desirable to consume enough folate to maintain maternal stores to keep pace with the increased demand resulting from marked cellular proliferation of maternal, placental and fetal tissues (Herbert 1987a). Moreover, compromised folate status has been linked to poor pregnancy outcomes (Goldenberg et al. 1992, Hibbard 1964, O’Scholl et al. 1996), although not in all investigations (Blot et al. 1981, Pritchard et al. 1972).

Supplemental folic acid, in addition to dietary folate, has been shown to prevent the negative folate status associated with pregnancy. Willoughby (1967) and Willoughby and Jewel (1968) illustrated that 300–350 μg/d synthetic folic acid in addition to diet (low folate content) was able to maintain normal folate status in pregnant women throughout gestation, whereas supplemental amounts of either 100 or 200 μg/d were inadequate. The discrepancy between these data and the findings of Chanarin’s group (1968b), in which the addition of 100 μg/d supplemental folic acid was adequate, may be explained by differences in dietary folate intake.

The role of folate in reducing the risk of neural tube defects (NTD) has been firmly established (Scott et al. 1995) and resulted in the U.S. Public Health Service recommendation that all women of childbearing age consume 400 μg/d of folic acid to reduce the risk of NTD (CDC 1992). Daly et al. (1995) subsequently demonstrated that RCF concentrations of 906 nmol/L (400 ng/mL) or higher were associated with a low risk of folate-responsive NTD. A study recently conducted by Brown et al. (1997) indicated that RCF concentrations ≥906 nmol/L (400 ng/mL) could be achieved by folate intakes of at least 450 μg/d (supplement users) or 500 μg/d (food and folic acid fortified cereals, only) provided that dietary estimates of folate consumption during the study period reflected folate consumption 2–3 mo earlier (during folate incorporation into reticulocyte).

This study was conducted to assess folate status in relation to highly controlled folate intake in pregnant adult women compared with that of nonpregnant controls. The study was designed to evaluate the adequacy of the current RDA for pregnant women (400 μg/d) (NRC 1989). Folate intakes were chosen to approximate the current and former RDA, 400 μg/d (NRC 1989) and 800 μg/d (NRC 1980), respectively. The folate status response of pregnant women was investigated throughout the second trimester of pregnancy as defined by Cunningham et al. (1993) because data suggest that this period of gestation may be associated with marked changes in folate utilization (McPartlin et al. 1993).

SUBJECTS AND METHODS

A two-by-two factorial study design was used in which pregnant women (n = 12) and nonpregnant controls (n = 12) were randomly assigned to consume folate intakes of either 450 μg/d (1020 nmol/d) or 850 μg/d (1926 nmol/d) for 84 d. The following four experimental groups (n = 6) were thus established: pregnant subjects fed either 450 or 850 μg/d folate and nonpregnant subjects fed the same two levels. Subjects consumed folate as a combination of dietary folate and synthetic folic acid taken with meals. A small percentage of the synthetic folic acid was provided as deuterated folic acid (1H) during the first half of the study, then switched to 100% unlabeled folic acid (1H), thus allowing for future investigation of folate kinetics (Fig. 1).

Blood and urine were collected at baseline and thereafter on a weekly basis for 12 wk (wk 14–25 gestation for pregnant subjects) as described below. In addition, after completion of the study, a subsample of our subjects participated in a 3-mo follow-up study during which they consumed either 200 μg/d (4 pregnant; 3 nonpregnant) or 600 μg/d (4 pregnant; 4 nonpregnant) synthetic folic acid plus uncontrolled dietary folate and returned to the Clinical Research Center for monthly blood draws.

Subjects. Healthy pregnant female subjects (18–35 y, 14 wk gestation) and nonpregnant controls (18–35 y) with normal blood chemistry profiles, normal blood folate concentrations and normal
Five-day cycle menus consumed by pregnant and nonpregnant subjects throughout 12-wk study\textsuperscript{1,2,3}

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
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</thead>
<tbody>
<tr>
<td><strong>Breakfast</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Waffles</td>
<td>Shredded wheat</td>
<td>Biscuits</td>
<td>Waffles</td>
<td>Shredded wheat</td>
</tr>
<tr>
<td>Margarine</td>
<td>Milk</td>
<td>Margarine</td>
<td>Margarine</td>
<td>Milk</td>
</tr>
<tr>
<td>Syrup</td>
<td>Raisins</td>
<td>Jelly</td>
<td>Syrup</td>
<td>Raisins</td>
</tr>
<tr>
<td>Cranberry juice</td>
<td>Brown sugar</td>
<td>Canned pears</td>
<td>Cranberry juice</td>
<td>Brown sugar</td>
</tr>
<tr>
<td>Apple sauce</td>
<td>Cranberry juice</td>
<td></td>
<td>Apple sauce</td>
<td>Cranberry juice</td>
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<tr>
<td><strong>Lunch</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sandwich,</td>
<td>Sandwich,</td>
<td>Crackers</td>
<td>Sandwich,</td>
<td>Sandwich,</td>
</tr>
<tr>
<td>Pita</td>
<td>Biscuit</td>
<td>Tuna</td>
<td>Pita</td>
<td>Biscuit</td>
</tr>
<tr>
<td>Turkey breast</td>
<td>Ham</td>
<td>Mayonnaise</td>
<td>Turkey breast</td>
<td>Ham</td>
</tr>
<tr>
<td>Cheese</td>
<td>Cheese</td>
<td>Chocolate chip cookies</td>
<td>Cheese</td>
<td>Cheese</td>
</tr>
<tr>
<td>Mayonnaise</td>
<td>Mustard</td>
<td>Cola</td>
<td>Mayonnaise</td>
<td>Mustard</td>
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<tr>
<td>Canned pears</td>
<td>Canned peaches</td>
<td></td>
<td>Canned pears</td>
<td>Canned peaches</td>
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<tr>
<td>Granola bar</td>
<td>Apple newtons</td>
<td></td>
<td>Granola bar</td>
<td>Apple newtons</td>
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<tr>
<td><strong>Dinner</strong></td>
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<tr>
<td>Casserole,</td>
<td>Turkey</td>
<td>Casserole,</td>
<td>Macaroni and</td>
<td>Chicken breast</td>
</tr>
<tr>
<td>Chicken</td>
<td>Rice</td>
<td>Ground beef</td>
<td>Cheese</td>
<td>Barbeque sauce</td>
</tr>
<tr>
<td>Rice</td>
<td>Gravy</td>
<td>Onion</td>
<td>Ground beef</td>
<td>Potato</td>
</tr>
<tr>
<td>Cheese soup</td>
<td>Green beans</td>
<td>Cream of mushroom soup</td>
<td>Margarine</td>
<td>Green beans</td>
</tr>
<tr>
<td>Margarine</td>
<td>Margarine</td>
<td>Margarine</td>
<td>Ginger snaps</td>
<td>Margarine</td>
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<tr>
<td>Green beans</td>
<td>Ice cream</td>
<td>Green beans</td>
<td></td>
<td>Green beans</td>
</tr>
<tr>
<td>Apple newtons</td>
<td>Chocolate syrup</td>
<td>Frozen juice flavored bar</td>
<td>Devi's food cookies</td>
<td>Devi's food cookies</td>
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<tr>
<td>Apple juice</td>
<td>Apple juice</td>
<td></td>
<td>Apple juice</td>
<td></td>
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<tr>
<td><strong>Snacks</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Oreo\textsuperscript{1} cookies</td>
<td>Oatmeal cookies</td>
<td>Graham crackers</td>
<td>Granola bar</td>
<td>Rice cakes</td>
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<tr>
<td>Milk</td>
<td>Milk</td>
<td>Yogurt</td>
<td>Milk</td>
<td>Milk</td>
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<tr>
<td>Yogurt</td>
<td>Yogurt</td>
<td>Popcorn</td>
<td>Yogurt</td>
<td>Yogurt</td>
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<tr>
<td>Popcorn</td>
<td>Popcorn</td>
<td></td>
<td>Popcorn</td>
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\textsuperscript{1}Menus analyzed by our laboratory provided 120 ± 15 μg/d (mean ± sd) folate.
\textsuperscript{2}Folate content of certain food items including green beans, white potatoes, chicken and ground beef was minimized by boiling three times.
\textsuperscript{3}Essential nutrients in the diet not meeting at least 90% of the RDA for either pregnant or nonpregnant women were provided in a custom-formulated supplement (Tishcon, Westbury, NY). See Table 2 footnotes for details.

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health status as determined by medical histories were eligible for participation. Exclusion criteria included chronic drug (including oral contraceptives and folate antagonists), alcohol or tobacco use. The majority of pregnant subjects (n = 10) were consuming prenatal vitamins containing folic acid (0.4–1.0 mg) before starting the study. Gestational age in pregnant subjects was determined by sonogram in conjunction with the first day of the last menstrual period. Approval of the study protocol by the Institutional Review Board of the University of Florida and signed informed consents by participants were obtained. Compliance to the study protocol was ensured by close daily contact in a positive environment monitored by the research team who observed consumption of folic acid with meals. Nonpregnant women maintained their body weight within 5% of baseline throughout the study and pregnant women gained ~0.45 kg/wk.

**Diet and supplements.** A 5-d menu cycle consisting of five dinners and three breakfasts and lunches was designed as detailed in Table 1. Conventional foods were selected to provide meals that were varied and palatable. Folate content was reduced by thrice boiling chicken, ground beef, green beans and white potatoes by using canned fruits and vegetables and refined starches. The menus were analyzed for their folate content in our laboratory and provided a mean of 120 ± 15 μg/d (272 ± 34 nmol/d). The combination of diet folate and supplemental folic acid provided total folate intakes of either 450 or 850 μg/d (120 μg dietary folate, 330 or 730 μg supplemental folic acid, respectively). Energy and all other nutrients were analyzed by the Minnesota Nutrient Data System version 2.7 (Nutrition Coordinating Center 1994). Table 2 includes the nutrient composition of the 5-d cycle menus. The menu cycle provided ~2500 kcal/d (10,467 kJ/d) of which ~60% came from carbohydrate, 25% from fat and 15% from protein. A custom-formulated supplement (Tishcon, Westbury, NY) was used to provide the RDA for all essential nutrients not met by the diet except folate. Loss of water-soluble vitamins/electrolytes in the boiled food items was accounted for and provided in the supplement when appropriate.

Energy intake was modified to maintain weight or ensure weight gain in nonpregnant and pregnant subjects, respectively. Examples of nonnutritive food sources included margarine, candy, Jello\textsuperscript{®}, Cool-Whip\textsuperscript{®} and sweetened or unsweetened beverages.

Commercially available folic acid (Sigma Chemical, St. Louis, MO) was used to prepare the folate supplements. Deuterated \( \text{[3,5-}^2\text{H}_4] \) folic acid was synthesized by the method of Gregory (1990), and proton nuclear magnetic resonance and HPLC were used to verify the purity and identity of these compounds before use. To prepare the supplement for consumption by subjects, the amount of deuterium labeled folic acid required to make the specific dose was weighed, dissolved in a small amount of 0.1 mol/L NaOH and brought to volume with food-grade sodium phosphate (dibasic) buffer adjusted to pH 7.0 with food-grade phosphoric acid. Unlabeled folic acid solutions in the desired quantities were similarly made. These stock solutions were divided into portions and stored at −70°C for use throughout the study. The volume of labeled and unlabeled folic acid solutions to be dispensed into each tube of apple juice was calculated.
TABLE 2

<table>
<thead>
<tr>
<th>Nutrient composition of 5-d cycle menus(^1,2,3)</th>
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<tbody>
<tr>
<td><strong>Day 1</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Energy, kJ</td>
</tr>
<tr>
<td>Protein, g</td>
</tr>
<tr>
<td>Fat, g</td>
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<tr>
<td>Folate, µg</td>
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</tbody>
</table>

1 At least 90% of the RDA for all essential nutrients was provided through the diet or supplementation.

2 Supplementation for pregnant subjects was provided by Tishcon (Westbury, NY): 0.4 mg thiamin mononitrate, 18 mg iron fumarate, 4.5 mg zinc sulfate, 230 retinol equivalent (RE) retinyl palmitate, 3 tocopherol equivalent (TE) d-a tocopheryl acetate, 3 µg cholecalciferol, 7 mg niacinamide, 1.5 mg pyridoxine HCl and 16 µg phylloquinone.

3 Supplementation for nonpregnant controls was provided by Tishcon (Westbury, NY): 3 mg iron fumarate, 1.5 mg zinc sulfate, 230 RE retinyl palmitate, 3 µg cholecalciferol, 5 mg niacinamide, 0.9 mg pyridoxine HCl and 14 µg phylloquinone.

RESULTS

Serum folate. Serum folate response for each experimental group throughout the 12-wk study is illustrated in Figure 2. At baseline, differences (P ≤ 0.05) existed between pregnant and nonpregnant women assigned to consume 450 µg/d (51 ± 19, 26 ± 17 nmol/L, respectively) and 850 µg/d (46 ± 22, 20 ± 10 nmol/L, respectively). No differences (P > 0.05) in baseline serum folate (SF) concentrations existed among pregnant women assigned to consume either 450 or 850 µg/d (51 ± 19, 46 ± 22 nmol/L, respectively) or among nonpregnant women assigned to consume these intakes (26 ± 17, 20 ± 10, respectively) (Fig. 2). At steady state, no differences (P > 0.05) were detected between pregnant and nonpregnant women consuming either 450 µg/d (27 ± 9, 26 ± 11 nmol/L, respectively) or 850 µg/d (43 ± 14, 42 ± 9 nmol/L, respectively) (Fig. 3). Differences (P ≤ 0.05) were found between pregnant subjects (27 ± 9, 45 ± 13 nmol/L) and nonpregnant subjects (26 ± 11, 43 ± 9 nmol/L) consuming 450 compared with 850 µg/d, respectively. All subjects maintained acceptable SF concentrations (>13.6 nmol/L) (Sauberlich et al. 1974) throughout the study period. Acceptable SF concentrations were also maintained throughout the follow-up period in pregnant and nonpregnant women consuming ~450 µg/d (34 ± 14, 26 ± 9 nmol/L, respectively) and ~850 µg/d (48 ± 14, 42 ± 21 nmol/L, respectively).
Red cell folate. Red cell folate response for each experimental group throughout the 12-wk study is illustrated in Figure 4. At baseline, differences (P ≤ 0.05) were not observed between pregnant and nonpregnant subjects assigned to consume either 450 µg/d (1383 ± 158, 1114 ± 397 nmol/L, respectively) or 850 µg/d (1174 ± 352, 767 ± 194, respectively). No differences (P > 0.05) were detected at baseline among pregnant subjects (1383 ± 158, 1174 ± 352 nmol/L) or nonpregnant controls (1114 ± 397, 768 ± 194.0 nmol/L) assigned to consume 450 compared with 850 µg/d, respectively (Fig. 4). At steady state, no differences (P > 0.05) existed between pregnant and nonpregnant women consuming 450 µg (1453 ± 252, 1000 ± 387 nmol/L, respectively) or between pregnant women consuming 450 compared with 850 µg/d (1453 ± 252, 1734 ± 209 nmol/L, respectively) (Fig. 5). Steady state was not achieved by the nonpregnant group consuming 850 µg/d within the time constraints of this study, and comparisons with this group could not be made. Final mean RCF concentration of the nonpregnant women consuming 850 µg/d (1283 ± 358 nmol/L) did not differ (P > 0.05) from any of the other groups. A positive correlation (r = 0.45; P = 0.03) was observed between RCF and SF and, had the study continued, differences between supplementation groups might have been detected. Acceptable RCF concentrations (>363 nmol/L) (Sauberlich et al. 1974) were maintained throughout the study period. In the subsample of subjects who participated in the follow-up study, RCF was also maintained within normal concentrations by pregnant and nonpregnant women consuming ~450 µg/d (1306 ± 272, 990 ± 238 nmol/L, respectively) and ~850 µg/d (1706 ± 220, 1246 ± 308 nmol/L, respectively).

**FIGURE 3** Serum folate concentration (group mean ± SD; n = 6) in pregnant and nonpregnant women consuming 450 or 850 µg/d folate at steady state. Bars designated by the same small letter were not significantly different (P > 0.05).

**FIGURE 4** Weekly mean red cell folate concentration (RCF, group mean; n = 6) in pregnant (P) and nonpregnant (NP) women consuming either 450 or 850 µg/d folate throughout the 12-wk study. Steady state was achieved at wk 1 for 450 µg/d nonpregnant group and at wk 1 and 7 for the 450 and 850 µg/d pregnant groups.

**FIGURE 5** Red cell folate concentration (RCF, group mean ± SD; n = 6) in pregnant (P) and nonpregnant (NP) women consuming either 450 or 850 µg/d folate at steady state. Final red cell folate concentration (mean ± SD; n = 6) is shown for the nonpregnant women consuming 850 µg/d folate because steady state was not achieved within the 12-week study. Bars designated by the same small letter were not significantly different (P > 0.05).

**FIGURE 6** Weekly urinary 5-methyl-tetrahydrofolate (THF) excretion (group mean; n = 6) in pregnant (P) and nonpregnant (NP) women consuming either 450 or 850 µg/d folate throughout the 12-wk study. Steady state was achieved at wk 1 and 6 for the 450 and 850 µg/d nonpregnant groups, respectively, and at wk 6 and 2 for the 450 and 850 µg/d pregnant groups.

**FIGURE 6** Weekly urinary 5-methyl-tetrahydrofolate (THF) excretion for each experimental group throughout the study period is illustrated in Figure 6. At baseline, differences (P ≤ 0.05) were observed between pregnant and nonpregnant women assigned to consume 450 µg/d (150 ± 240, 31 ± 40 nmol/d, respectively) or 850 µg/d (359 ± 120, 43 ± 91 nmol/d, respectively). Differences (P ≤ 0.05) in baseline 5-methyl-THF excretion were not detected among pregnant (150 ± 240, 359 ± 120 nmol/d) or nonpregnant women (31 ± 40, 43 ± 91 nmol/d) assigned to consume 450 compared with 850 µg/d, respectively, largely as a result of the enormous variability in the urinary excretion of this metabolite (Fig. 6). At steady state, no differences (P > 0.05) were detected between pregnant and nonpregnant women consuming 450 µg/d (10 ± 3, 15 ± 11 nmol/d, respectively) or 850 µg/d (198 ± 100, 146 ± 59 nmol/d, respectively). Differences (P ≤ 0.05) were ob-
served at steady state between pregnant women (10 ± 3, 198 ± 101 nmol/d) and nonpregnant controls (11 ± 11, 146 ± 26 nmol/d) consuming 450 compared with 850 µg/d, respectively (Fig. 7). Urinary 5-methyl-THF was positively correlated with SF (r = 0.74; P = 0.0001) and RCF (r = 0.27; P = 0.21).

**Urinary folic acid.** At the end of the 84-d protocol, folic acid was not being excreted by either of the 450 µg/d groups or by the 850 µg/d pregnant group. Final mean urinary folic acid excretion of the 850 µg/d nonpregnant group was 33.0 ± 28.6 nmol/L (19% of total urinary folate; 2% folic intake). Concentrations were also maintained above 906 nmol/L (400 ng/mL) throughout the study and follow-up period. Thus, pregnant women were excreting similar amounts of 5-methyl-THF (136 nmol/d, respectively) at steady state, no detectable folic acid. Pregnant women consuming 450 compared with 850 µg/d nonpregnant group consumed either 450 or 850 µg/d supplemental folic acid was sufficient to maintain mean RCF concentrations above 906 nmol/L (400 ng/mL) throughout the study and follow-up period. Thus, pregnant women consumed 850 µg/d throughout the study and follow-up period.

Although urinary excretion of folate is not considered to be a reliable index of folate status (Sauberlich et al. 1987), one might expect to observe differences between pregnant and nonpregnant women and between supplementation groups. These differences may reflect metabolic differences, thereby enhancing the information gained from blood indices. Pregnant women have been found to excrete significantly more folate than nonpregnant women, which is hypothesized by some investigators to contribute to the increase in requirements during pregnancy (Fleming 1972, Landon and Hytten 1971). Pregnant and nonpregnant women consuming 450 µg/d were excreting similar amounts of 5-methyl-THF (～113 nmol/d at steady state and no detectable folic acid. Pregnant and nonpregnant women consuming 850 µg/d were also excreting similar amounts of 5-methyl-THF (～180 compared with 136 nmol/d, respectively) at steady state, ～15-fold higher than the 450 µg/d groups. Only the 850 µg/d nonpregnant group excreted folic acid (19% of total urinary folate). Saleh et al. (1980) reported a lower folic acid to 5-methyl-THF ratio in patients with malignant disease (state of increased cellular proliferation) compared with controls. They suggested that malignant disease increased the demand for folate and led to more rapid metabolism of folic acid to the reduced folate pool as indicated by an increase in 5-methyl-THF excretion relative to folic acid. Their explanation may apply to our finding that only nonpregnant women in the 850 µg/d group excreted unmetabolized folic acid because pregnancy also represents a period of increased cellular proliferation. The higher urinary excretion of 5-methyl-THF (metabolized form) in the 850 µg/d groups may reflect the saturable process of folate reabsorption from glomerular filtrate by proximal tubular cells (Williams and Huang 1982). Overall, these data on urinary folate excretion indicate that pregnant women are not excreting more

**DISCUSSION**

This 12-wk metabolic study was designed to assess the adequacy of the current (400 µg/d) and former (800 µg/d) RDA for folate in pregnant women. This is the first controlled metabolic study conducted to investigate folate status response of pregnant women to defined folate intakes. Pregnant women during their second trimester (wk 14–25) and nonpregnant controls consumed either 450 or 850 µg/d as synthetic folic acid and dietary folate. Group means for various folate status indices were compared at steady state.

Serum folate concentration is considered a sensitive index of recent folate status (Herbert 1987b) because it is highly influenced by current dietary intake. However, under metabolic conditions, in which dietary intake is constant, SF concentration should reflect the overall folate status of the individual. The rapid decline in SF concentration in the pregnant women consuming 450 µg/d illustrates the SF response to a lower folate intake. Once acclimated to this lower intake, SF concentration was maintained within normal limits in the pregnant group consuming 450 µg/d and was equivalent to the nonpregnant controls at the same supplementation level. Hemodilution did not appear to be a factor in the initial decline in SF concentration because the 850 µg/d pregnant group did not experience any decline throughout the 12-wk period and steady-state SF concentrations were equivalent to those of the 850 µg/d controls. Hemodilution or expansion of blood volume is a known physiologic consequence of pregnancy (Cunningham et al. 1993), and a significant decline in hematocrit values from baseline was observed in the pregnant women participating in this study. It can be assumed, therefore, that the pregnant women had higher total amounts of SF at steady state than the nonpregnant controls.

Red cell folate concentration reflects liver folate concentration and is considered to be an indicator of long-term folate status (Herbert 1987b). Folate is accumulated only by developing reticulocytes (Shane 1995); because the life span of red cells is about 120 d, RCF concentration more accurately reflects folate status 2–3 mo before the time of analysis. However, because red cells are being synthesized daily over a 12-wk period, one should be able to detect some change in concentration. This is especially true during pregnancy when red cell production increases by ～33% (Blackburn and Loper 1992), resulting in greater changes in RCF concentration if inadequate amounts of folate are available at the time of incorporation. In our pregnant group consuming 450 µg/d, RCF was maintained throughout the study period and was equivalent to the 450 µg/d control group at steady state. Perhaps more importantly, normal RCF concentrations were also maintained throughout the 3-mo follow-up period in the subsample of subjects who returned for subsequent blood draws. In addition, 450 µg/d (food folate + supplemental folic acid) was sufficient to maintain mean RCF concentrations above 906 nmol/L (400 ng/mL) throughout the study and follow-up period. Thus, supporting the findings of Brown et al. (1997). Red cell folate concentrations were also maintained above 906 nmol/L in both the pregnant and nonpregnant women consuming 850 µg/d throughout the study and follow-up period.

FIGURE 7 Urinary 5-methyl-tetrahydrofolate (THF) excretion (mean ± SD; n = 6) in pregnant and nonpregnant women consuming either 450 or 850 µg/d folate at steady state. Bars designated by the same small letter were not significantly different (P > 0.05).
folate than nonpregnant women and support the blood data in which no differences between pregnant and nonpregnant women within the same supplementation group were detected. Homocysteine, a functional index of folate status, was also quantified but will be reported separately.

From these data and within the constraints of our study, we conclude that 450 µg/d derived from both food (120 µg) and synthetic folic acid (330 µg) is adequate to maintain normal folate status in well-nourished pregnant women during their second trimester. However, there remains the question of translating this finding into dietary equivalents because the overall goal was to provide metabolic data to assist in the interpretation of the current RDA. Recent studies (Cuskelly et al. 1996, Pfeiffer et al. 1997) indicate that folic acid in fortified food is highly available unlike naturally occurring food folate. Therefore one may hypothesize that synthetic folic acid consumed with meals is more available than endogenous food folate (~50%) (Sauberlich et al. 1987) but less available than the essentially complete absorption of synthetic folic acid (~100%) consumed under fasting conditions (Gregory 1995). A reasonable, although somewhat conservative approach is to assume that synthetic folic acid consumed with meals is ~75% available. Based on the above assumptions, our subjects in the 450 µg/d group consumed ~307 µg/d available folate which translates into 615 µg/d dietary equivalents. Our dietary equivalent estimate resembles the estimations of Chanarin et al. (1968a and 1968b) and McPartlin et al. (1993). It also supports the recommendation of Willoughby et al. (1968) regarding the administration of 300–350 µg/d synthetic folic acid throughout gestation, assuming an average folate intake of 150 µg/d (low dietary folate). It is questionable whether the majority of pregnant women can consume ~600 µg/d of folic acid from diet alone (Brown et al. 1997, Huber et al. 1988, LSRO 1980), although folic acid enrichment of cereal-grain foods (140 µg/100 g product) effective January 1, 1998 (FDA 1996) is estimated to increase average daily consumption by 80–100 µg (Brown et al. 1997, FDA 1996). Our findings suggest that prenatal vitamins containing more than 500 µg/d are not necessary to maintain adequate folate status in well-nourished pregnant populations and support the findings of Lowenstein et al. (1966), which suggested that provision of 500 µg/d synthetic folic acid, in addition to low dietary folate intake, may be above the minimal daily requirement because higher serum folates were observed in pregnant women compared with nonpregnant controls (24.9 vs. 15.9 nmol/L, respectively). Because the effects of oversupplementation with folate on the developing fetus are unknown (Scott et al. 1991), high supplemental doses (>1000 µg/d) should be avoided under normal circumstances. The first and third trimesters or postpartum periods were not investigated under controlled conditions in the current study; therefore, future areas of research should encompass these time frames under controlled conditions as well as the response of previously unsupplemented pregnant women to defined folate intakes.

ACKNOWLEDGMENTS
The authors wish to acknowledge and thank Jeff Opalko for assistance with blood folate analyses; Gail Kauwell for use of HPLC equipment; and Rita Harris for nursing supervision. We also thank the staff at the Clinical Research Center for assistance during this protocol.

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