Folate Catabolism in Pregnant and Nonpregnant Women with Controlled Folate Intakes

Marie A. Caudill,* Jesse F. Gregory III,* Alan D. Hutson† and Lynn B. Bailey*†

*Food Science and Human Nutrition Department, University of Florida, Gainesville, FL 32611 and †Division of Biostatistics, Department of Statistics, University of Florida, Gainesville, FL 32610

ABSTRACT Measurement of the urinary folate catabolites, para-aminobenzoylglutamate (pABG) and the more predominant acetylated form, acetamidobenzoylglutamate (apABG), has been used to assess folate requirements in both pregnant and nonpregnant women. Folate catabolite excretion has been reported to be significantly higher in pregnant women (second trimester) compared with nonpregnant controls. The primary goals of this study were to determine if pregnant women in a controlled metabolic study excreted higher quantities of urinary folate catabolites than nonpregnant controls and if catabolite excretion was influenced by folate intake. We evaluated the effect of gestation and folate intake on the urinary excretion of apABG and pABG in pregnant women (n = 12; wk 14–26 gestation) and nonpregnant controls (n = 12) assigned to consume folate levels approximating the current (400 µg/d) and previous (800 µg/d) RDA. Subjects were fed a controlled diet containing 120 µg folate/d and either 330 or 730 µg synthetic folic acid/d. In contrast to previously reported data, no differences in mean folate catabolite excretion were detected between pregnant and nonpregnant subjects. Catabolite excretion (pABG + apABG) decreased significantly relative to initial values in pregnant women consuming 450 µg folate/d (−40 ± 20%; mean ± se) and final mean excretion was significantly lower in the pregnant women consuming 450 µg folate/d (86 ± 32 nmol/d) compared with 850 µg folate/d (148 ± 20 nmol/d). Data from this study indicate that second trimester pregnant women do not excrete more folate catabolites than nonpregnant controls and that consumption of 450 vs. 850 µg folate/d results in a significant reduction in the quantity of folate catabolites excreted. J. Nutr. 128: 204–208, 1998.

KEY WORDS: • folate • requirements • catabolites • pregnancy • humans

Folate coenzymes are integral components of one carbon metabolism and are necessary for the synthesis of DNA, RNA and certain amino acids (Wagner 1995). Pregnancy is associated with an enormous increase in cellular proliferation due to increased maternal erythropoiesis, maternal uterine and mammary tissue growth as well as placental and fetal development (Blackburn and Loper 1992, Cunningham et al. 1993). The increased demand for folate during pregnancy, often resulting in compromised folate status, is well established (Bailey et al. 1980, Chanarin et al. 1968, Colman et al. 1975, Ek and Magnus 1981, Lowenstein et al. 1966, Qvist et al. 1986) and is one of few nutrients for which the Recommended Dietary Allowance (RDA) doubles from nonpregnant conditions (NRC 1989). During the past 60 y, explanations for the higher folate requirements have included increased need for folate coenzymes involved in cell division (Hibbard 1964), malabsorption (Giles 1966), increased urinary folate excretion (Fleming 1972, Landon and Hytten 1971), fetal drainage (Ek and Magnus 1981, Giles 1966), and, most recently, increased folate catabolism (McPartlin et al. 1993). The finding that only 1–2% of folate intake is excreted as intact urinary folate suggests that folate is broken down before urinary excretion (Luke et al. 1947). Studies conducted in rats (Connor et al. 1979, Murphy et al. 1976 and 1978, Scott and Gregory 1996) and humans (Blair et al. 1957, Krumdieck et al. 1978, Pheasant et al. 1979, Saleh et al. 1980) have confirmed that the major route of whole body folate turnover is by catabolism and cleavage of the C9-N10 bond producing pteridines and para-aminobenzoylglyutamate (pABG). Before excretion from the body, the majority of pABG is N-acetylated to acetamidobenzoylglutamate (apABG). McPartlin et al. (1993) quantitated the urinary excretion of the major catabolites, pABG and apABG, in pregnant women at one time point during each trimester and once in nonpregnant controls. They reported that pregnant women in the second trimester excreted twice as much urinary apABG as nonpregnant controls or pregnant women in the first trimester. A modest increase in apABG excretion also was observed in the third trimester. Recommended dietary allowances were computed by combining both catabolites to folate equivalents.
on the basis that the molecular weight of either catabolite is approximately one-half that of intact folate (McPartlin et al. 1993). The small quantity of urinary intact folate (2.3–22.7 nmol/d) was added to this value, and the total was adjusted for bioavailability and population variance. The estimated RDA based on the urinary excretion of folate catabolites and intact folates corresponded to 280 µg folate/d (nonpregnant and first trimester), 660 µg folate/d (second trimester) and 470 µg folate/d (third trimester). The decrease in folate catabolism observed during the third trimester was hypothesized to represent a change in the nature of growth from hyperplastic to hypertrophic (McPartlin et al. 1993). The current RDA for pregnant women is 400 µg folate/d (NRC 1989), which was decreased by 50% from the former RDA of 800 µg folate/d (NRC 1980). The work of McPartlin et al. (1993) suggests that the current RDA is inadequate. Potential limitations of the study conducted by McPartlin and associates (1993) include uncontrolled dietary folate intake between hospital admissions and the fact that the catabolites were measured at only one time point during each trimester.

We examined the urinary excretion of pABG and apABG in pregnant women (n = 12) during their second trimester and in nonpregnant controls (n = 12) throughout a 12-wk period during which time folate intake was controlled rigidly. One objective of this study was to determine if differences existed in urinary pABG and apABG excretion between pregnant and nonpregnant subjects. A second objective was to determine if differences existed in urinary catabolite excretion between pregnant women consuming 450 compared with 850 µg folate/d. The folate status response of the subjects also was monitored by measuring the concentrations of serum and red cell folate, urinary 5-methyl-THF, urinary folic acid and plasma homocysteine. These results are reported separately (Caudill et al. 1997; Bonnête R. E. et al., unpublished data).

**MATERIALS AND METHODS**

The protocol was approved by the University of Florida’s Institutional Review Board and informed consents were obtained from all subjects. Healthy pregnant subjects (n = 12; 14–26 wk gestation; 18–35 y) and nonpregnant controls (n = 12; 18–35 y) were assigned randomly to folate intakes of either 450 or 850 µg/d (1020 or 2126 nmol/d, respectively). Pregnant women participated in this study from their 14th to 26th wk of pregnancy, which represents this trimester (14–28 wk) as defined by Cunningham et al. (1993). Gestational age was determined by sonogram in conjunction with date of last menstrual period. Four experimental groups (n = 6) were compared: pregnant subjects fed either 450 or 850 µg folate/d and nonpregnant subjects fed the same two levels. These intakes approximated the current (400 µg folate/d) and former (800 µg folate/d) RDA for pregnant women (NRC 1980 and 1989). The diet consumed by all participants consisted of conventional foods as described previously (Caudill et al. 1997) and provided 120 ± 15 µg folate/d. The remainder, either 330 or 730 µg folate/d, was provided as synthetic folic acid administered in apple juice and consumed with meals (Caudill et al. 1997). Considering the higher bioavailability of synthetic folic acid consumed with food (~75%) relative to dietary folate (~50%), the two levels of intake equate to ~600 and 1000 µg/d dietary equivalents (Caudill et al. 1997). The RDA for all essential nutrients was provided as a combination of diet and a custom formulated supplement (Tishcon, Westbury, N.Y.). All participants consumed breakfast, dinner and supplements at the University of Florida Clinical Research Center, whereas lunch and snacks were consumed away from the research center.

The body mass index (BMI) of pregnant (prepregnancy weights) and nonpregnant women was within normal range 19–26 kg/m² with means ± SD of 22 ± 3 and 23 ± 2 kg/m², respectively. Energy intake was adjusted by manipulation of non-nutritive food items such as sweetened gelatin, margarine, candy and sweetened or unsweetened beverages to achieve weight maintenance and weight gain in nonpregnant and pregnant subjects, respectively. The pregnant subjects gained ~0.45 kg/wk, resulting in an average weight gain of 5.2 ± 1.2 kg throughout the second trimester. Nonpregnant women achieved weight maintenance.

Weekly 24-h urine collections were obtained in 2-L brown plastic jugs containing 3 g sodium ascorbate. Subjects were instructed to keep urine refrigerated at all times during collection to protect against bacterial growth. After recording total urine volumes, urine was mixed thoroughly, dispensed into 200-mL portions and stored at ~20°C.

**Isolation and quantification of apABG and pABG.** Urinary pABG and apABG were isolated and quantified by the method of McPartlin et al. (1992) with minor modifications. Urinary catabolites were quantified in duplicate on a bimonthly basis in pregnant women (weeks 0, 2, 4, 6, 8, 10 and 12) and on a monthly basis in nonpregnant controls (weeks 0, 4, 8 and 12).

Urine samples (50–200 mL) were adjusted to pH 7.0, filtered through Whatman number 1 filter paper and passed through affinity chromatography columns containing immobilized folate binding protein to remove intact folates (Gregory and Toth 1988, Selhub et al. 1980). Because recovery tests indicated complete recovery of both pABG and apABG from the folate affinity columns, the radioactive standards were added after this step. The effluent from the affinity column was next separated into duplicate 20-mL portions to which [3H]pABG and [3H]apABG were added. The 20-mL samples were adsorbed to a final concentration of 0.1 mol/L HCl and applied to glass columns (200 × 15 mm) filled to a height of 5.0 cm with a slurry of Dowex 50W (50X8-400, Sigma Chemical, St. Louis, MO) cation exchange resin equilibrated with 0.1 mol/L HCl. Acetamidobenzoylglutamate was eluted in the sample volume and the following 50 mL 0.1 mol/L HCl effluent, which were pooled and retained. A second 0.1 mol/L HCl wash was applied (higher concentrations resulted in pABG elution) and discarded. Para-aminobenzoylglutamate was eluted and collected in 100 mL of 0.6 mol/L HCl. The fraction containing apABG was acidified to 0.2 mol/L HCl and heated at 100°C for 60 min to deacetylate apABG. The deacetylated pABG fraction then was cooled to ambient temperature, adjusted to 0.1 mol/L HCl and reapplied to re-equilibrated ion exchange columns. After a 100-mL wash with 0.1 mol/L HCl, pABG representing apABG was eluted and collected in 100 mL 0.6 mol/L HCl. The two RDA fractions were kept separate throughout the remainder of the purification and quantification process. The 100-mL fractions containing pABG and apABG were derivatized by the method of Bratton and Marshall (1939). One milliliter of 50 mol/L HCl and 1 mL sodium nitrite solution (1%) were added to each sample and allowed to react for 5 min at room temperature. Ammonium sulfamate solution (50 g/L; 1 mL) then was added to destroy excess HNO₃. After 5 min, 1 mL N-(1-naphthyl)ethylendiamine solution (10 g/L) was added to couple with pABG. The samples set overnight at room temperature to allow complete formation of the purple-colored azo-N-(1-naphthyl)-ethylendiamine derivative of pABG (azo-pABG). Each sample (~104 mL) was applied to a preactivated (5 mL methanol wash followed by 5 mL H₂O) C₁₈ Sep Pak cartridge (Waters, Milford, MA) under negative pressure for further purification and concentration. After a 10 mL 0.05 mol/L HCl wash, azo-pABG was eluted in 4 mL 100% methanol. The solution was evaporated to dryness at 40°C under a stream of nitrogen. The residue was reconstituted in 250 μL H₂O, and pABG was regenerated upon addition of 25 μL HCl (5 mol/L) and 25 μl zinc dust suspension (1 g/3 mL H₂O). After 15 min of intermittent mixing, the reaction mixture was transferred to a 1-mL Eppendorf tube and centrifuged for 10 min. The supernatant was removed, filtered to remove zinc particles and stored at ~20°C before HPLC analysis. Recovery of [3H]pABG and [3H]apABG were ~80 and 60%, respectively, and were the values used to correct the final estimates of catabolites.

The amount of pABG (representing the two catabolites) in a sample was determined by reverse-phase HPLC under the following conditions: 100-μL injection volume, octadecylsilica column (Ultrasphere C₁₈, 5-μm particle size; 4.6 mm I.D. × 250 mm; Phenomenex, Torrance, CA), flow rate 1.0 mL/min, isocratic mobile phase composed of acetonitrile (2%) and 0.1 mol formic acid/L (98%), pH 4.0. A UV absorption detector ( Dionex AD20) monitoring at 280 nm was
used for detection of pABG. Quantification of pABG (representing excreted pABG and apABG) was accomplished relative to standards ranging in concentration from 100 to 1500 ng/mL prepared from commercial pABG (Sigma Chemical) using a published molar absorb-  tivity (Blakley 1969) to determine the concentration of the pABG in stock solution. Intraassay and interassay coefficients of variation were 10 and 8%, respectively, based on the addition of tritiated standards to 10 pooled urine samples analyzed on 10 different days. Tritiated pABG and apABG were synthesized from [3H]folic acid (Amersham, Arlington Height, IL) as described by McPartlin et al. (1992) and were used as internal standards. In 50% of the duplicate urine samples. The fact that our duplicates varied <10% and that we consistently recovered 60 ± 10% [3H]pABG and 80 ± 10% [3H]apABG confirmed the accuracy and reproducibility of this method.

Statistical analyses.} Compared were made between groups for differences in initial, final and percent change = 100 × (final-baseline)/baseline means. In addition, trends were examined using nonlinear regression fitting the model of the form \( \mu = \beta_0 [1 - \exp(-\beta_1 \text{wk})] \) and tested using the same slopes linear test for trend. All tests of means were performed at \( \alpha = 0.05 \) using ANOVA (SAS/STAT Version 6.12, SAS Institute, Cary, NC). Contrasts were examined using Tukey’s multiple comparison procedure. Pearson correlations were used to describe associations between urinary apABG and pABG concentrations and other folate status indices. Values in tables, figures and text are means ± SD.

RESULTS

Urinary apABG. Initial (d1), final (d84) and percent change at the end of the 84-d experimental period from initial d1 measurement for apABG excretion in pregnant and nonpregnant women consuming either 450 or 850 μg folate/d are shown in Table 1. No differences (\( P > 0.05 \)) were detected in initial means for any of the groups. Urinary apABG excretion decreased (\( P = 0.059 \)) over time at a rate of about 4.4 nmol/wk in the pregnant women consuming 450 μg folate/d and resulted in significantly less (\( P = 0.05 \)) apABG excretion by pregnant women consuming 450 vs. 850 μg folate/d at the end of the experimental period (Table 1). Figure 1 illustrates the pattern of change over time in the different groups. No differences were detected in final mean apABG excretion between nonpregnant women consuming 450 vs. 850 μg folate/d. No differences were detected in final means between pregnant and nonpregnant groups regardless of level of supplementation.

Urinary pABG. Initial (d1), final (d84) and percent change at the end of the 84-d experimental period from initial d1 measurement for pABG excretion in pregnant and nonpregnant women consuming either 450 or 850 μg folate/d are illustrated in Table 2. Pregnant women assigned to consume 850 μg folate/d excreted more pABG at baseline compared with nonpregnant controls and experienced a rapid decline in pABG excretion during the first 2 wk of the study (Figure 2). The excretion of pABG decreased significantly (\( P < 0.05 \)) over time at about 1.4 nmol/wk in pregnant subjects consuming 450 μg folate/d and was less at the end of the experimental period than in the 850 μg folate/d pregnant group (Table 2). In contrast, no difference (\( P > 0.05 \)) in final mean pABG excretion was detected between nonpregnant women consum-
ing 450 vs. 850 µg folate/d. No differences in final means were detected between pregnant and nonpregnant women regardless of level of supplementation. Urinary pABG was correlated positively with serum folate \( r = 0.42, P = 0.04 \) and urinary 5-methyl-tetrahydrofolate \( r = 0.52, P = 0.001 \) in contrast to apABG, which was not significantly correlated with these indices.

**Urinary total pABG + apABG.** The fact that pregnant women did not excrete higher quantities of folate catabolites relative to nonpregnant controls also was illustrated by the total urinary folate catabolite excretion (pABG + apABG) (Table 3). In contrast to an expected increase in folate catabolite excretion during the course of the second trimester, total folate catabolite excretion significantly decreased over time in the pregnant subjects consuming 450 µg folate/d.

**DISCUSSION**

The urinary folate catabolites, pABG and the more predominant form apABG, were measured at numerous time points throughout a 12-wk period during which time pregnant subjects in their second trimester (wk 14–26) and nonpregnant controls had folate intakes of either 450 or 850 µg/d. The primary focus was differences in initial and final mean catabolite excretion and changes in catabolite excretion with time.

Our results did not confirm the work of McPartlin et al. (1993), who reported a twofold higher urinary apABG excretion by pregnant women during the second trimester relative to nonpregnant controls. We found that pregnant women consuming 450 µg folate/d experienced a decline in apABG excretion from baseline measurements \((-37.8 \pm 20.4\%\) that was not observed in the 450 µg folate/d nonpregnant group. Although differences between pregnant and nonpregnant women consuming 450 µg folate/d were not detected in final group means, the study continued or had the subject number been larger, differences may have been observed. Significant differences were detected in final mean apABG excretion between pregnant women consuming 450 vs. 850 µg folate/d, and these differences were not detected between nonpregnant subjects consuming these amounts. These data indicate that gestation is associated with a reduction in the urinary excretion of apABG when folate intake is somewhat lower (i.e., 450 vs. 850 µg/d) and suggests that pregnant women may be more efficient at conserving folate than their nonpregnant counterparts.

A decline in the urinary excretion of pABG \((-49 \pm 25\%\) also was observed for the pregnant women consuming 450 µg folate/d that was not observed in the 450 µg folate/d nonpregnant group. In addition, significant differences were detected in final mean pABG excretion between pregnant women consuming 450 vs. 850 µg folate/d but not between the nonpregnant 450 or 850 µg folate/d groups. These findings suggest that pABG excretion is also affected by gestation during periods of lower intake (450 vs. 850 µg folate/d) and may be the result of reduced endogenous folate catabolism or less excretion of intact folate, which could later degrade to pABG.

Although our findings in pregnant women differed from that repeated by McPartlin’s group, agreement existed in catabolite values of nonpregnant women among this study and those of McPartlin et al. (1993) and Kownacki-Brown et al. (1995). The mean urinary apABG and pABG concentrations in our study were \(-110 and 27 nmol/d, respectively, compared \(-104 and 49 nmol/d, respectively, (McPartlin et al. 1993) and \(-150 nmol/d (apABG + pABG) before supplementation (Kownacki-Brown et al. 1995). The differences between our findings in apABG excretion during pregnancy and those of McPartlin et al. (1993) may have been influenced by the differences in protocols. In the present study, folate intake was highly controlled for a 12-wk period during which time repeated 24-h urine collections were obtained. In contrast, folate intake only was controlled for a 42-h period in the study by McPartlin et al. (1993).

The concept of deriving requirements based solely on catabolite excretion appears to be inappropriate during pregnancy. In addition to the apparent ability of pregnant women to conserve folate when intake is limited, other avenues of folate loss or accretion should be considered. For example, red cell production increases by 33% during pregnancy (Blackburn and Loper 1992), resulting in greater folate uptake by reticulocytes. This extra demand for folate will not be reflected in urinary catabolite concentration. The doubling in uterus size may require more folate coenzymes of which the majority may be recycled upon utilization and not degraded and excreted as urinary catabolites. The developing fetus drains maternal folate supplies as evidenced by the significantly higher plasma and red cell folate concentrations in newborns compared with maternal concentrations (Ek and Magnus 1981, Giles 1966). Folate accumulated by the placenta and delivered to the developing fetus will not be estimated by measurement of maternal folate catabolites. In addition, measurement of urinary catabolite excretion alone does not account for endogenous fecal loss of intact folates or catabolites, which may be substantial (Krumdieck et al. 1978).

In conclusion, these data indicate that urinary folate catabolite excretion does not increase during the second trimester of pregnancy and therefore cannot explain the increased requirement for folate by pregnant women. In contrast, data from this study indicate a reduction in urinary apABG and pABG excretion during pregnancy in response to lower folate intakes (i.e., 450 vs. 850 µg/d) and thus implies pregnant women may be more efficient at conserving folate. Future areas of research include investigation of catabolite excretion at numerous time points during the first and third trimesters and postpartum periods under conditions in which folate intakes and other confounding variables are strictly controlled.

**TABLE 3**

<table>
<thead>
<tr>
<th>Group</th>
<th>Folate intake</th>
<th>Baseline (µg/d)</th>
<th>Final (µg/d)</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/d</td>
<td></td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Pregnant 450</td>
<td>153 ± 52</td>
<td>86 ± 32</td>
<td>-40 ± 20</td>
<td></td>
</tr>
<tr>
<td>Nonpregnant 450</td>
<td>120 ± 39</td>
<td>115 ± 37</td>
<td>4 ± 42</td>
<td></td>
</tr>
<tr>
<td>Pregnant 850</td>
<td>178 ± 61</td>
<td>148 ± 20</td>
<td>-3 ± 52</td>
<td></td>
</tr>
<tr>
<td>Nonpregnant 850</td>
<td>116 ± 31</td>
<td>138 ± 31</td>
<td>22 ± 25</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 6, differences in columns (P ≤ 0.05) are noted by superior a or b.

**LITERATURE CITED**
