Urinary Excretion of Folate Catabolites Responds to Changes in Folate Intake More Slowly than Plasma Folate and Homocysteine Concentrations and Lymphocyte DNA Methylation in Postmenopausal Women¹,²

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ABSTRACT Folate turnover involves urinary excretion, fecal excretion, and catabolism that involves cleavage of the C9-N10 bond to yield pterins and para-aminobenzoyleglutamate (pABG). Little is known about the relationship between the function of folate pools and their rates of catabolism. We report here an investigation of excretion of urinary pABG and its primary excretory form, para-acetamidobenzoyleglutamate (ApABG) in samples collected during a previously published study of postmenopausal women. Ten women (49–63 y) were fed a low folate diet (56 μg/d) supplemented with folic acid to yield total folate intakes of 195 μg/d (d 1–5), 111 μg/d (d 6–41), 286 μg/d (d 70–80) and 516 μg/d (d 81–91). This caused changes in plasma folate, plasma homocysteine and global methylation of lymphocyte DNA. For each subject, a 7-d pooled urine sample was collected over d 1–7, 36–42, 64–70 and 85–91. ApABG constituted >85% of total catabolite excretion, and folate intake did not significantly influence ApABG or pABG excretion. The molar ratio of total catabolite excretion/folate intake varied significantly, with ratios of 1.0 ± 0.17 (d 1–7), 3.0 ± 0.55 (d 36–42), 1.1 ± 0.18 (d 64–70) and 0.33 ± 0.054 (d 85–91). These observations indicate that the rate of folate catabolite excretion is related mainly to masses of slow-turnover folate pools governed by long-term folate intake. Folate pools functioning in some forms of methyl group metabolism respond to dietary changes in folate intake much more rapidly. J. Nutr. 130: 2949–2952, 2000.

KEY WORDS: • folate • catabolism • pterins • para-aminobenzoyleglutamate • para-acetamidobenzoyleglutamate • humans

The role of a catabolic process in folate metabolism has long been recognized. Dinning et al. (1957) first reported the excretion of free and acetylated diazotizable amines after administration of folic acid or 5-formyltetrahydrofolate in rats. Subsequent studies indicated that the catabolic process involved cleavage of the folate C9-N10 bond, with the primary excretory products identified as various pterins (Krumdieck et al. 1978), and the folate-derived amines including para-aminobenzoyleglutamate (pABG) and its N-acetyl derivative, para-acetamidobenzoyleglutamate (ApABG) (Barford et al. 1978, Murphy et al. 1976, Murphy and Scott 1979). The urinary excretion of ApABG greatly exceeds that of nonacetylated pABG (Caudill et al. 1998, Geoghegan et al. 1995, McNulty et al. 1987, McPartlin et al. 1992). Acetylation appears to be catalyzed by an arylamine N-transferase isoform with specificity toward pABG (Estrada-Rodgers et al. 1998, Minchin 1995).

The relationship between folate nutritional status and the rate of pABG and ApABG excretion has not been fully established, although excretion of pABG and/or ApABG is not clearly indicative of folate nutritional status. In addition, it is unclear which specific form(s) of folate and body pools are most prone to catabolism. Urinary excretion of folate catabolites greatly exceeds the excretion of intact folate in rats (Wang et al.1994) and humans (Caudill et al. 1998, Gregory et al. 1998). At higher intakes of folate, urinary folate excretion increases to a proportionally greater extent than does catabolite excretion. Supplementation with folic acid has little effect on the short-term excretion of folate catabolites (Kowacki-Brown et al. 1993, McNulty et al. 1987). In studies of chronic control of folate intake, the excretion of folate catabolites is proportional to folate intake (Caudill et al. 1998, Gregory et al. 1998). In a study in which nonpregnant women were chronically given oral doses of deuterated fol acid, examination of the rate and extent of labeling of ApABG provided evidence that folates from both rapid- and slow-turnover pools are subject to catabolism (Gregory et al. 1998). For all intakes examined (200, 300 and 400 μg total folate/d), ApABG excretion greatly exceeded the excretion of intact folate. The sum of pABG and ApABG, along with catabolites, appears to account for the majority of folate catabolites. Geoghegan et al. (1995) concluded that the excretion of ApABG is itself a suitable indicator of folate catabolism. Folate catabolism also may be increased by anti-convulsant drugs, alcohol and certain cancers (Kelly et al. 1979 and 1981, Shaw et al. 1989).

Measurement of total excretion of folate catabolites (i.e., ApABG + pABG) has been reported in a number of studies.

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² Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

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McPartlin et al. (1993) examined catabolite excretion in free-living pregnant women and nonpregnant controls. They reported that nonpregnant controls, postpartum women and women in the first trimester of pregnancy excreted total catabolites that were the equivalent of \( \sim 100 \) \( \mu \)g of folate/d, which corresponded to \( \sim 227 \) nmol/d. Greater excretion of catabolites was reported during the second and third trimesters of pregnancy. Long-term dietary control was not employed in that study. Caudill et al. (1998) conducted a similar study except that folate intake was precisely controlled for 12 wk in second trimester pregnant women and nonpregnant controls. In this protocol, no difference was observed between pregnant and nonpregnant women consuming the same level of folate (either 450 or 850 \( \mu \)g/dl). However, the steady-state excretion of total pABG + ApABG increased in proportion to folate intake. The study of Caudill et al. (1998) showed clearly that at least 4 wk was required during these relatively high levels of controlled intake.

Little is known about the level of folate catabolism and time required for catabolite excretion to stabilize during periods of low dietary folate intake. In this paper, we report the measurement and interpretation of pABG and ApABG excretion in a study with carefully controlled dietary intake intended to provide depletion followed by repletion with folate. This provided an ideal context for examination of effects of a modest folate deficiency on catabolite excretion. Detailed analysis of the influence of dietary intake on various functional indices of folate status has been reported in a separate publication (Jacob et al. 1998).

**SUBJECTS AND METHODS**

**Protocol.** As described previously (Jacob et al. 1998), healthy nonsmoking postmenopausal volunteer women (\( n = 10 \), ages 49–63 y, were admitted to the metabolic unit of the USDA Western Human Nutrition Research Center (WHNRC) after medical and psychological screening. Normal renal function was ensured by testing for serum creatinine, blood urea nitrogen and urinalysis at screening and biweekly throughout the study. Tests for plasma folate and vitamin B-12, alcohol, tobacco and drug use were also performed. All subjects were within 90±30% of desirable weight (Metropolitan Life Insurance Company, New York, NY), except for one subject who was 145% of desirable weight. Other criteria for subject selection included the following: nonsmokers, nonusers of vitamin or other dietary supplements containing folic acid, hemoglobin > 115 g/L and hematocrit > 0.34. Three subjects, who were receiving estrogen replacement therapy when they entered the study, continued the same regimen throughout the study. The study protocol and informed consent were approved by the Human Subjects Review Committee of the University of California, Davis, and by the Human Studies Review Committee of the Agricultural Research Service, U. S. Department of Agriculture. Signed informed consent was obtained from each volunteer. For the duration of the 91-d study, the subjects lived in and ate all meals in the WHNRC metabolic unit and were chaperoned at all times when outside the unit.

Throughout the 91-d study, a low folate, low choline diet was fed, which provided an average of 56 \( \mu \)g/d folate from food sources (Jacob et al. 1999). Synthetic folic acid (pteroylglutamic acid) was added to the diet at various stages of the protocol to allow precise control of folate intake from 56 to 516 \( \mu \)g/d. Total folate intakes were as follows: 195 \( \mu \)g/d (d 1–5), 56 \( \mu \)g/d (d 6–41), 111 \( \mu \)g/d (d 42–69), 286 \( \mu \)g/d (d 70–80) and 516 \( \mu \)g/d (d 81–91).

**Specimen collections and analytical methods.** Blood samples were obtained weekly, and complete urine collections were obtained as successive 7-d pools for each subject. All samples were stored at \(-70^\circ\)C until analyzed. Urinary pABG and ApABG were measured using the procedure of McPartlin et al. (1992) with modifications as described by Caudill et al. (1998). Excretion per day of pABG and ApABG was calculated by multiplying the concentration of each compound in a given pooled urine sample by the mean daily urine volume. We analyzed four urine pools corresponding to study d 1–7 (baseline), 36–42, 64–70 and 85–91. Urine collections at d 36–42 and 64–70 represent the end of each depletion period (56 and 111 \( \mu \)g/d folate intake, respectively), whereas d 85–91 represent the end of the repletion period (11 d at 286 \( \mu \)g/d, then 11 d at 516 \( \mu \)g/d). However, the folate intakes corresponding to each of the first three urine pools are not exactly the same as the dietary folate intakes of the preceding period because the 7-d urine pool periods overlapped the succeeding dietary folate intake period by 2, 1 and 1 d, respectively. Hence, the average folate intakes during each of the first three 7-d urine pools were 155, 58, and 117 \( \mu \)g/d, rather than 195, 56, and 111 \( \mu \)g/d. The average folate intake during the last 7-d urine pool is the weighted average of 10 d at 286 \( \mu \)g/d and 11 d at 516 \( \mu \)g/d, a weighted average of 406 \( \mu \)g/d.

Additional measurements reported here were made using methods described in the previous paper regarding this study (Jacob et al. 1998), including the following. Plasma homocysteine was measured by fluorometric HPLC (Araki and Sako 1987). The methyl acceptor capacity of lymphocyte DNA was measured by a modification of the method of Balaghi and Wagner (1993), in which the extent of SssI CpG methylase-catalyzed transfer of \([3H]\)methyl groups from [methyl-\(^3\)H]S-adenosylmethionine is reflective of the relative state of hypomethylation of available sites. Plasma folate was measured by competitive protein binding radioassay (Quanaphase II B-12/Folate Radioassay, BioRad, Hercules, CA). Total folate content of the diet was determined by microbiological assay as previously described (Jacob et al. 1998).

**Statistical analysis.** The effects of folate intake on the excretions of each catabolite in the respective dietary treatment periods were determined by one-way repeated-measures ANOVA (Glanz 1992). Further analysis of data, including molar ratios of ApABG/(pABG + ApABG) and (pABG + ApABG)/total folate intake, was also conducted by one-way repeated-measures ANOVA. Differences were considered significant at \( P < 0.05 \). When significance was observed, multiple comparisons were conducted using the Student-Newman-Keuls method. Statistical analyses were conducted using SigmaStat for Windows version 1.0 (Jandel Corporation, San Rafael, CA).

**RESULTS**

The measurement of urinary folate catabolites yielded the results presented in Table 1. Excretions of pABG and ApABG did not change significantly during the experiment. Summarizing observations for all samples collected throughout this protocol, the subjects excreted 308 ± 27.5 nmol pABG/d and 253 ± 2.48 nmol pABG/d (means ± SEM, \( n = 40 \) total observations). Total folate catabolite excretion (i.e., pABG + ApABG) was 333 ± 28.4 nmol/d (mean ± SEM, \( n = 40 \) total observations), which corresponds to daily elimination of 147 ± 12.5 \( \mu \)g folic acid equivalents through catabolism throughout this study. The excretion of ApABG constituted 89.9 ± 1.50% of total pABG + ApABG excretion (mean ± SEM, \( n = 40 \) total observations), and this value did not change significantly throughout the various dietary periods of the study.

We also investigated the relationship between excretion and folate intake. The molar ratio of total urinary pABG + ApABG/folate intake varied markedly (\( P < 0.0001 \)) over the course of the study (Table 1). This molar ratio rose from 1.05 ± 0.17 during the initial dietary period (155 \( \mu \)g/d folate intake) to 3.0 ± 0.55 during the 58 \( \mu \)g/d depletion period (means ± SEM, \( P < 0.0001 \)). In other words, folate catabolite excretion exceeded folate intake by a factor of \( \sim 3 \) during wk 5 of 58 \( \mu \)g/d folate intake.

**DISCUSSION**

Although the very substantial role of folate catabolism as a component of folate turnover has been demonstrated...
pABG excretion, nmol/d
pABG + ApABG excretion, nmol/d
ApABG/(pABG + ApABG), mol/mol
(pABG + ApABG)/folate intake, mol/mol
Plasma folate, nmol/L
Plasma homocysteine, μmol/d
Lymphocyte DNA methyl acceptance, cpm/0.5 μg DNA

TABLE 1
Folate catabolite excretion, plasma folate and biochemical indices of one-carbon metabolism in postmenopausal women during a protocol with various controlled dietary folate intakes1,2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pooled urine sample</th>
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</thead>
<tbody>
<tr>
<td>Days of protocol</td>
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<tr>
<td>Period of controlled folate intake</td>
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<tr>
<td>1–5 (baseline)</td>
<td></td>
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<tr>
<td>6–41 (depletion)</td>
<td></td>
</tr>
<tr>
<td>42–69 (depletion)</td>
<td></td>
</tr>
<tr>
<td>70–91 (repletion)</td>
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<tr>
<td>Folate intake during each dietary period, μg/d (nmol/d)</td>
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<tr>
<td>7-d urine pool, μg/d (nmol/d)</td>
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<tr>
<td>ApABG excretion, nmol/d</td>
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<tr>
<td>pABG excretion, nmol/d</td>
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<tr>
<td>pABG + ApABG excretion, nmol/d</td>
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<tr>
<td>ApABG/(pABG + ApABG), mol/mol</td>
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<tr>
<td>(pABG + ApABG)/folate intake, mol/mol</td>
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<tr>
<td>Plasma folate, nmol/L</td>
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<tr>
<td>Plasma homocysteine, μmol/d</td>
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<tr>
<td>Lymphocyte DNA methyl acceptance, cpm/0.5 μg DNA</td>
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</table>

1 Means ± SEM, n = 10, except n = 8 for plasma folate, homocysteine and DNA methyl acceptance. Values for catabolites are means of 7-d urine pools collected at the end of each treatment period. Because of slight overlap of urine collection between diet periods, average intakes for each urine collection are shown. These values represent, for each protocol period, average folate intake during and prior to the urine collection. Data for plasma folate, homocysteine and DNA methyl acceptance are for samples collected at the end of each treatment period. Abbreviations: pABG, para-aminobenzoylglutamate; ApABG, para-acetamidobenzoylglutamate.

2 Means are different: *from baseline d 5; ‡ from previous mean, P < 0.001.

3 Values followed by different superscript letters are different, P < 0.0001.

4 Previously reported by Jacob et al. (1998), presented here to facilitate comparison with data regarding excretion of folate catabolites.

Previously, little is known about the body folate pool(s) that undergoes catabolism. Jacob et al. (1998) reported data from this study indicating that plasma homocysteine and methylation status of lymphocyte DNA underwent significant changes during the course of the dietary manipulations of this protocol. These findings contrast with the observations reported here, indicating that folate catabolism is only weakly influenced by short-term changes of folate intake. Thus, we infer that folate pools that serve as precursors of folate catabolites differ from those directly involved in cellular acquisition and generation of one-carbon units, as 5-methyl-tetrahydrofolate, destined for homocysteine remethylation and formation of S-adenosylmethionine for cellular methylation reactions. The results of this study are consistent with the findings of Caudill et al. (1995) who reported that the excretion of folate catabolites increased slowly after the initiation of folate supplementation in pregnant women and nonpregnant controls.

The calculation of the molar ratio of catabolites and folate intake is not meant to imply that there is a direct temporal linkage between recently ingested folate and excreted catabolites. By contrast, the molar ratio of ~3 after 5 wk of 58 μg/d folate intake demonstrates clearly that it was the tissue folate derived from previous intake that yielded the excreted catabolites. This is consistent with the observation of Kownacki-Brown et al. (1993), who found that only a small percentage of a bolus oral dose of deuterated folate appeared in short-term collections of urine. Under the conditions of this protocol, it is likely that the overall turnover of body folate would occur very slowly, probably with an overall mean residence time for total body folate of ~200 d as reported by Gregory et al. (1998). This is consistent with the lack of significant change in red cell folate, which is known to turn over much more slowly than the 5-methyl-tetrahydrofolate pool. The relatively rapid responsiveness of plasma homocysteine, and lymphocyte DNA methylation status reported by Jacob et al. (1998) suggests that 5-methyl-tetrahydrofolic acid responsible for these functional changes of one-carbon metabolism is in a much greater state of flux than the bulk of the body folate pools, which turn over much more slowly. Several abundant enzymes are known to bind folate very tightly, which may account for a portion of the large, relatively refractory component of body folate. Further work is required to identify such pools and their in vivo behavior. An additional finding of interest is the fact that the proportion of total pABG that undergoes acetylation of the aryl amino group is relatively constant and not influenced by the level of folate intake. It should be noted that the diet used in this study was low in choline; thus, it is likely that low choline intake accentuated the methyl group deficiency caused by folate deficiency (Jacob et al. 1999). One should also recognize that not all aspects of methyl group metabolism are impaired during folate deficiency. In a previous study of short-term folate depletion coupled with low methionine and choline intakes in men, plasma homocysteine concentration increased, whereas methylation of a test dose of nicotineamide (as an indicator of methylation capacity) was unchanged (Jacob et al. 1995).

In summary, the findings reported here illustrate clear kinetic differences between folate pools functioning directly in methyl group metabolism and those from which folate catabolites are primarily generated. From the point of view of the design of nutrition intervention studies regarding folate, this study adds to the growing body of evidence that most studies of practical duration do not achieve a steady state of folate status or metabolism. Hence, such intervention studies must be interpreted cautiously regarding effects of relatively short-term dietary treatments. However, these observations also indicate that clear changes in plasma folate concentration and aspects of methyl group metabolism occur well before complete attainment of steady state of all tissue folate pools.

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LITERATURE CITED


