

Folate catabolite excretion is responsive to changes in dietary folate intake in elderly women¹⁻⁴

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ABSTRACT

Background: The major route of folate turnover is by catabolic cleavage of the C9-N10 bond producing *p*-aminobenzoylglutamate (pABG) and its primary excretory form, *p*-acetamidobenzoylglutamate (ApABG). We hypothesize that total pABG (ApABG + pABG) excretion parallels both the mass of body folate pools from which these catabolites originate and the folate-status indicators.

Objective: The objective was to determine whether urinary folate catabolite excretion reflects body pool size and parallels the static and functional measures of folate status.

Design: Urinary folate catabolite excretion was measured in women (aged 60–85 y) consuming controlled amounts of folate for 14 wk. A low-folate diet (120 µg/d) was consumed ($n = 33$) for 7 wk, and then subjects were randomly assigned to consume either 200 ($n = 14$) or 400 ($n = 16$) µg folate/d. Urinary pABG and ApABG concentrations were measured by HPLC at 0, 7, and 14 wk.

Results: Urinary excretion of total pABG was significantly lower ($P = 0.001$) after depletion (73.9 ± 4.7 nmol/d) than at baseline (115 ± 12.7 nmol/d). This rate of decline ($\approx 0.7\%$ per day) is consistent with the kinetically measured rate of turnover of total body folate at moderate folate intakes. The average percentage increase in total pABG in response to folate repletion with 400 µg/d (75%) was significant ($P = 0.02$). Folate catabolite excretion was significantly ($P = 0.0001$) associated with serum and red blood cell folate, plasma homocysteine, and DNA hypomethylation after depletion and with serum folate ($P = 0.001$) and plasma homocysteine ($P = 0.0002$) after repletion with 400 µg folate/d.

Conclusions: Total urinary pABG excretion reflects total body folate pool size and is a long-term indicator that parallels functional measures of folate status. *Am J Clin Nutr* 2003;77:919–23.

KEY WORDS Folate status, folate catabolites, elderly women, folate depletion, folate repletion, *p*-aminobenzoylglutamate, *p*-acetamidobenzoylglutamate

INTRODUCTION

The existence of a catabolic process in the metabolism of folate was first identified by Dinning et al (1), who detected the urinary excretion of free and conjugated diazotizable amines derived from folates administered to rats. This catabolic process involves cleavage of the C9-N10 bond of folates, as indicated by urinary excretion of various pterins derived from folate cleavage in humans (2) and of folate-derived amines including *p*-aminobenzoylglutamate (pABG) and the acetylated form, *p*-acetamidobenzoylglutamate

(ApABG) (3–5). Urinary excretion of ApABG exceeds that of free pABG (6–9). Although other products may exist, studies in rodents suggest that pABG and ApABG are the largest urinary products of folate catabolism (4,5). Urinary excretion of total pABG in humans greatly exceeds that of intact folate (7, 10), except at folate intakes > 400 µg/d (9). Compartmental modeling of folate metabolism suggests both that folate catabolism is a constitutive component of the turnover of tissue folate pools and that the rate of urinary excretion of folate catabolites would be proportional to the total body folate pool size (10).

There is much interest in identifying sensitive biomarkers of normal folate status that may be used to evaluate the effects of physiologic changes (eg, age and pregnancy) and environmental influences (eg, alcohol and drugs). This issue is of particular interest in the elderly because it is recognized that inadequate folate intake is inversely associated with chronic disease risk, as recently reviewed (11). The current knowledge of the folate intake required to maintain normal folate status in the elderly is primarily derived from observational data in population-based studies (12).

This metabolic study is the first to investigate whether urinary folate catabolite excretion is sensitive to changes in controlled folate intake (14 wk) and whether there is an association between folate catabolite excretion and other folate status indicators in women aged > 63 y. In addition, the hypothesis that urinary folate catabolite excretion is an indicator of body folate pool size was evaluated by observing the effect of low folate intake on the rate of decline in folate catabolite excretion and comparing that finding with estimates of whole-body folate turnover rates derived from previous studies of folate kinetics.

SUBJECTS AND METHODS

Subjects

Thirty-three healthy women aged 60–85 y ($\bar{x} \pm \text{SEM}$: 71.6 ± 1.1 y) completed the 7-wk depletion phase of the study, and 30 subjects

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completed the entire 14-wk protocol. Potential subjects were screened by telephone and personal interviews and then with the use of a physical examination and blood chemistry profile. Exclusion criteria included abnormal blood chemistry, history of chronic disease, abnormal renal function as determined from age-adjusted creatinine-clearance values, body weight > 120% of ideal, chronic alcohol consumption, the use of tobacco products, and the use of any prescription medication including estrogen-replacement drugs. At baseline, overall means for serum folate (47.2 ± 4.7 nmol/L), red blood cell (RBC) folate ($1.9 \pm 1.3 \times 10^2$ nmol/L), pyridoxal phosphate (61.1 ± 5.1 nmol/L), vitamin B-12 (462 ± 30 pmol/L), and homocysteine (Hcy) (9.51 ± 4.9 μ mol/L) concentrations were within normal limits (ie, ≥ 7 nmol/L, ≥ 317 nmol/L, ≥ 20 nmol/L, ≥ 130 pmol/L, and < 14 μ mol/L, respectively). The University of Florida Institutional Review Board approved the study, and written informed consent was obtained from each subject.

Experimental design and diet

The 14-wk study was divided into 2 periods of 49 d (7 wk) each. Subjects were outpatients in the General Clinical Research Center, and they consumed all of the foods provided to them and only those foods and beverages. Personal monitoring by the research team at meal times and weekly serum folate measurements were used to ensure compliance. At the time the study was designed, dietary folate intake was not expressed as dietary folate equivalents (DFEs); however, average DFEs are provided in parentheses for comparison. During the first 7 wk of the study (depletion phase), subjects consumed a low-folate diet providing 118 (rounded to 120) μ g folate/d (120 μ g DFEs/d) as described previously (13). Subjects were randomly assigned to 1 of 2 dietary treatment groups during the 7-wk repletion phase of the study, and those groups received either 200 or 415 (rounded to 400) μ g folate/d (231 or 568 μ g DFEs/d). The basal diet fed throughout the study included a 5-d menu cycle comprising foods that were typical of a mixed diet and were consumed with folic acid mixed in apple juice during the repletion phase. The folate concentration of the diet was measured with the use of a modification of the trienzyme extraction method of Martin et al (14) and was analyzed with the use of the microplate adaptation of the *Lactobacillus casei* microbiological assay (15, 16). The diet provided an average energy intake of 7929 kJ (1895 kcal/d; 13% protein, 65% carbohydrate, and 22% fat). A daily custom-formulated multivitamin supplement (Tishcon Corp, Westbury, NY) and a separate iron supplement (18 mg Fe/d; General Nutrition Center, Pittsburgh) were provided to ensure that the intake of all nutrients other than folate met the recommended daily allowance. The nutrient profile of the study diet for all nutrients except folate was estimated with the use of the MINNESOTA NUTRIENT DATA SYSTEM software, version 2.7 (Nutrition Coordinating Center at the University of Minnesota, Minneapolis). To maintain body weight at $\pm 5\%$ of each subject's initial weight, minor dietary adjustments were made by substituting higher- or lower-energy versions of menu items.

Sample collection

Each week, one complete 24-h urine sample was collected in 2-L brown plastic containers with 3 g sodium ascorbate and kept refrigerated during collection to prevent the growth of bacteria. After total urine volume was recorded, portions were stored in a frozen state at -30°C . Blood samples were collected on the same

day as the 24-h urine samples. Creatinine clearance was calculated from serum and urinary creatinine concentrations as a means of monitoring the completeness of urine collections. Changes in folate status were monitored by measurement of serum and RBC folate concentrations, plasma Hcy concentration, and DNA methylation as previously reported (13, 17).

Catabolite assay

Urinary catabolites (pABG and ApABG) were isolated and quantified in duplicate at baseline and at weeks 7 (after depletion) and 14 (after repletion) by the method of McPartlin et al (7) with minor modifications. As performed by Caudill et al (9), each sample was passed through a folate-binding affinity chromatography column to remove urinary folate, thus eliminating this potential interference in the assay of the catabolites.

Quantification of pABG was made relative to standard curves prepared from commercial pABG (Sigma Chemical Co, St Louis) with the use of the published molar absorptivity (18). Catabolite recovery was monitored by the addition of tritiated pABG and ApABG to selected urine samples before sample purification and HPLC analysis (7). The concentrations of pABG and ApABG in urine samples were adjusted for the mean recovery values. The method was also verified by the observation that pABG was fully resolved from other peaks in each HPLC chromatogram. Intra-assay and interassay CVs were 10% and 8%, respectively, according to the addition of tritiated standards to 10 pooled urine samples analyzed on 10 different days.

Statistical analysis

Initial descriptive statistics and baseline variables were evaluated by analysis of variance to verify that no significant differences existed between the groups at baseline. Analysis of covariance was used to evaluate group differences in total pABG at week 7 (adjusting for week 0 values) and at week 14 (adjusting for week 7 values). Values are presented as means \pm SEMs.

During the depletion and repletion phases of the study, a direct relation between folate catabolite excretion and other indicators of folate status—serum and RBC folate concentrations and DNA methylation status—was expected (ie, it was expected that all indicators would decrease with folate depletion and increase with folate repletion). Conversely, an inverse relation between folate catabolite excretion and plasma Hcy concentration was expected in response to folate depletion and repletion. The sign test for proportion of trends was used as a secondary outcome to evaluate the relation of trends for 2 variables (eg, total pABG and serum folate) over time (eg, weeks 0 and 7). Regression analysis was used to determine the slope for each subject during the depletion phase (week 0 to week 7) and the repletion phase (week 7 to week 14) for total pABG in relation to the slopes for the other previously determined indicators including DNA methylation, Hcy concentration, and serum and RBC folate concentrations. The signs of the slopes (positive or negative) were then tallied, and the observed proportion was tested against the proportion that could be expected by chance alone [ie, there are 4 possible trends (+/+, +/-, -/+, and -/-), and the proportion of any possible combination is 25% or 0.25] with the use of a sign test for trends. In addition to the sign test for trends, simple correlations between total pABG excretion and other variables were examined by Pearson's correlation coefficients. Differences were considered significant at $P < 0.05$. All statistics were computed with the use

TABLE 1

Excretion of *p*-aminobenzoylglutamate (pABG), *p*-acetamidobenzoylglutamate (ApABG), and total pABG (ApABG + pABG) at baseline (week 0) and after depletion (week 7) and subsequent repletion (week 14) of folate¹

Folate catabolite	Baseline (<i>n</i> = 42)	Week 7 (<i>n</i> = 33)	Week 14	
			Repletion with 200 µg folate/d (<i>n</i> = 14)	Repletion with 400 µg folate/d (<i>n</i> = 16)
pABG (nmol/d)	20.5 ± 2.8	13.6 ± 1.0 ²	18.3 ± 2.9	24.6 ± 3.0 ³
ApABG (nmol/d)	94.1 ± 11.0	60.3 ± 4.1 ⁴	50.3 ± 6.8	81.9 ± 7.7 ⁵
Total pABG (nmol/d)	115 ± 12.7	73.9 ± 4.7 ⁶	68.6 ± 9.2	106.5 ± 9.9 ⁷

¹ $\bar{x} \pm \text{SEM}$.

^{2,4,6}Significantly different from baseline: ² $P < 0.05$, ⁴ $P = 0.006$, ⁶ $P = 0.001$.

^{3,5,7}Significantly different from week 7: ³ $P = 0.0017$, ⁵ $P = 0.0113$, ⁷ $P = 0.0031$.

of SAS software, version 8.0 (SAS Institute, Cary, NC), and all *P* values were based on two-sided tests.

RESULTS

No significant differences ($P > 0.05$) were detected in post-depletion values in subjects randomly assigned to either of the treatment groups (ie, 200 or 400 µg/d, respectively) for age (70.8 ± 1.8 or 72.5 ± 1.3 y), weight (61.3 ± 2.3 or 64.1 ± 2.3 kg), serum (12.1 ± 1.7 or 15.6 ± 1.6 nmol/L) (13) and RBC (13.4 ± 1.2 × 10² or 16.5 ± 1.6 × 10² nmol/L) folate concentrations, DNA methylation status (34.4 ± 0.95 × 10³ or 31.8 ± 0.87 × 10³ decays/min) (17), plasma Hcy concentration (11.0 ± 0.7 or 11.6 ± 1.2 µmol/L) (13), and concentrations of pyridoxal phosphate (55.3 ± 5.2 or 67.7 ± 9.8 nmol/L), vitamin B-12 (400 ± 23 or 499 ± 55 pmol/L), pABG (13.7 ± 1.3 or 13.4 ± 1.4 nmol/d), ApABG (60.1 ± 4.8 or 60.5 ± 7.0 nmol/d), and total pABG (73.8 ± 5.4 or 73.9 ± 8.0 nmol/d). Hematocrit values did not change significantly over the 14-wk study.

Mean pABG, ApABG, and total pABG values for week 0 (baseline), week 7 (after depletion), and week 14 (after repletion) are shown in **Table 1**. Mean urinary excretion of total pABG (pABG + ApABG) was significantly ($P = 0.0001$) lower after depletion (73.9 ± 4.7 nmol/d) than at baseline (115 ± 12.7 nmol/d). The 7-wk low-folate diet resulted in a 35.7% decline in total pABG excretion, which corresponds to a rate of decline of 0.73% per day.

A significant difference ($P = 0.001$) in the mean percentage change (20%) in total pABG excretion from baseline to after depletion was detected and was primarily due to a reduction in ApABG, with a mean percentage change of 19.6% compared with 3.6% for pABG. In contrast with the consumption of 400 µg folate/d, when 200 µg folate/d was consumed during repletion, total pABG continued to decrease (mean percentage change: 13%; $P > 0.05$). The mean percentage change in catabolite excretion from the depletion phase to the repletion phase in the group consuming 200 µg folate/d did not differ significantly (pABG, $P = 0.165$; ApABG, $P = 0.2597$; and total pABG, $P = 0.6255$). In contrast, in response to repletion with 400 µg folate/d, the mean percentage change for total pABG excretion differed significantly (75%; $P = 0.02$) from week 7 to week 14, as reflected in the significantly higher mean concentrations of pABG, ApABG, and total pABG at week 14 than at week 7 (Table 1).

The changes observed in total pABG, serum and RBC folate concentrations, plasma Hcy concentrations, and DNA methylation status from baseline (week 0) to after depletion (week 7) and

repletion (week 14) in the group consuming 400 µg folate/d are shown in **Figure 1**. With the use of the sign test for trends analysis, a significant direct relation ($P = 0.0001$) was detected between total pABG and serum and RBC folate concentrations and DNA methylation status during the depletion phase (week 0 to week 7). Total pABG and serum and RBC folate concentrations simultaneously decreased in 76% of the subjects, and total pABG concentration and DNA methylation both decreased in 61% of the subjects. In addition, a significant ($P = 0.0001$) inverse relation between total pABG and plasma Hcy concentration for week 0 to week 7 was observed in 73% of subjects.

In response to folate repletion (week 7 to week 14) with 400 µg/d, a significant ($P = 0.003$) direct relation between total pABG and serum folate concentration was observed. In addition, a significant ($P = 0.013$) inverse relation with plasma Hcy concentration was detected. In contrast, no significant relation ($P > 0.05$) between total pABG excretion and RBC folate concentration or DNA methylation status was observed during repletion.

At baseline, significant positive correlations were observed between total pABG and serum folate ($r = 0.44$, $P = 0.009$) and RBC folate ($r = 0.54$, $P = 0.001$) concentrations. In addition, an inverse correlation was detected at baseline between total pABG and plasma Hcy concentration ($r = -0.41$, $P = 0.018$). At the end of the depletion period (week 7), significant correlations between total pABG and RBC folate ($r = 0.55$, $P = 0.001$) concentrations and between total pABG and plasma Hcy ($r = -0.60$, $P = 0.001$) concentrations were observed. After the repletion period (week 14), total pABG was significantly correlated with serum folate concentration when the two repletion groups (200 and 400 µg/d) were combined ($r = 0.57$, $P = 0.001$) and with RBC folate ($r = 0.41$, $P = 0.023$) and plasma Hcy ($r = -0.42$, $P = 0.020$) concentrations. Within the 400 µg/d repletion group, total pABG was inversely correlated with plasma Hcy concentration ($r = -0.72$, $P = 0.002$).

DISCUSSION

The significant reduction in urinary folate catabolites in response to the low-folate diet and the different responses to dietary folate intakes of 200 and 400 µg/d support the assumption that total urinary folate catabolite excretion is sensitive to changes in folate intake. To date, only one other study has been conducted to evaluate the effect of controlled folate intake on urinary folate catabolite excretion in older (aged 49–63 y) women (19). In contrast to the present study, the earlier study detected no significant change in urinary folate catabolite excretion in response to

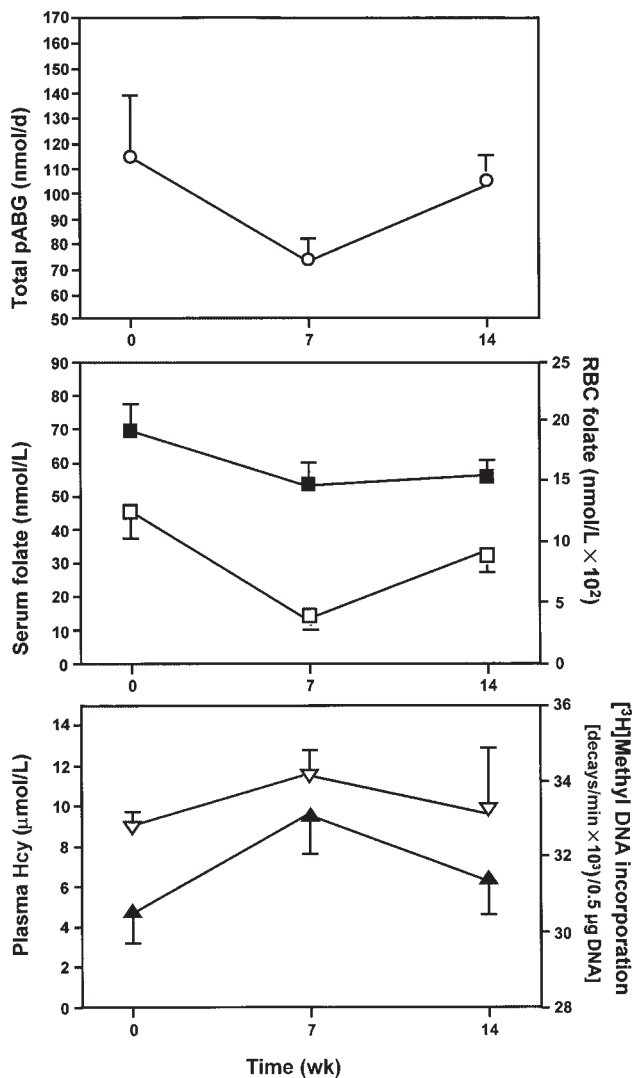


FIGURE 1. Mean (\pm SEM) concentrations of total *p*-aminobenzoylglutamate (pABG), serum and red blood cell (RBC) folate, and plasma homocysteine (Hcy) and mean (\pm SEM) DNA methylation status at baseline (week 0) and after depletion (week 7) and subsequent repletion (week 14) of folate in the subjects assigned to consume 400 μ g folate/d ($n = 16$). Data were reported previously for serum and RBC folate and plasma Hcy concentrations (13) and for DNA methylation status (17). The total pABG concentration at week 7 was significantly ($P < 0.05$) different from that at baseline and from that at week 14. The RBC folate (■) concentration at week 7 was significantly ($P < 0.05$) different from that at baseline, and the serum folate (□) concentration at week 7 was significantly ($P < 0.05$) different from that at baseline and from that at week 14. The plasma Hcy (▽) concentration at week 7 was significantly ($P < 0.05$) different from that at baseline and from that at week 14, and [³H]methyl DNA (▲) incorporation at week 7 was significantly ($P < 0.05$) different from that at baseline.


changes in folate intake. The duration of the dietary treatment periods in the earlier study (≈ 2 –4 wk) may not have been sufficient for detection of differences in urinary folate catabolite excretion in response to a folate depletion-repletion protocol (20). This explanation is supported by very short-term studies in which folic acid supplementation had little effect on the excretion of folate catabolites (6, 21). Gregory et al (10) reported a kinetic model of

folate metabolism based partially on ApABG excretion in young women in response to the consumption of either 200, 300, or 400 μ g folate/d for 10 wk. That model indicated that the rate of folate turnover increased in proportion to intake in young women. The predicted proportion of tissue folate pools that underwent catabolism per unit of time was only weakly affected by folate intake. Conversely, the rate of folate catabolism was mainly a function of tissue folate pool size. In the present study, folate catabolite excretion was significantly reduced in response to the low-folate diet, which may reflect a decrease in tissue folate catabolism or in the rate of folate turnover. As stated previously, the 7-wk low-folate diet resulted in a 35.7% decline in mean total folate catabolite excretion, which corresponds to a rate of decline of 0.73% per day. Kinetic analysis of whole-body folate turnover in young adult women with folate intakes of 200–400 μ g/d indicated turnover rates of $\approx 0.5\%$ and 0.8% per day, respectively (10). The similarity between the rate of decline in folate catabolite excretion in the dietary depletion phase of the present study and the turnover rates for whole-body folate determined previously strongly suggests that folate catabolite excretion is an indicator of body folate pool size.

The significant association with other well-established predictors of folate status—including serum and RBC folate concentrations, plasma Hcy concentration, and DNA methylation status—suggests that folate catabolite excretion is reflective of folate status. It has been suggested that urinary folate catabolites relate to the masses of the slower-turnover body folate pools rather than to those that function in methyl group metabolism (19), which supports our conclusion that the duration of the protocol in this study was sufficient for detection of changes in these slower body folate pools. The identification of a folate status indicator that is reflective of changes in tissue folate pools is an important finding because other commonly used folate status indicators may not be as sensitive or specific, as recently reviewed (11). For example, because folate uptake by the erythrocyte occurs only at the reticulocyte stage, the interpretation of RBC folate concentrations is hindered by the long lag time associated with responses to changes in dietary folate intake (11). Although the serum folate concentration changes quickly in response to dietary changes, it may or may not indicate the status of tissue stores (11). Plasma Hcy concentration and DNA methylation status have been referred to as “functional” indicators of folate status that may be indicative of the inadequacy of methyl groups associated with inadequate folate intake (11). The fact that significant differences were not detected between DNA methylation after folate repletion and DNA methylation after folate depletion suggests that leukocyte DNA methylation may be slow to respond to changes in folate status relative to blood folate concentrations (17). Changes in Hcy or DNA methylation may reflect a generalized methyl group deficiency and are not totally specific for folate inadequacy (11).

The folate catabolite data from this study of healthy elderly subjects at baseline and after repletion with 400 μ g folate/d are very similar to previously reported data (total pABG = ≈ 110 nmol/d) for young adult subjects (9, 22, 23). These data suggested that folate catabolite excretion responds to changes in dietary folate intake in a similar manner in both old and young persons within the controlled research conditions of a metabolic protocol, and they support the measurement of folate catabolites as a folate status indicator in the elderly. An important limitation of the measurement of folate catabolites is the fact that the analytic method is tedious and time-consuming, which makes it undesirable for use

in large-scale, population-based studies. Improvements in this method, such as are reported by Higgins et al (24), may allow extended assessment of folate catabolite measurements.

In conclusion, data from the present study suggest that folate catabolite excretion is an indicator of body folate pool size, and they support the use of urinary folate catabolite excretion as a folate status indicator. As such, it may be used with other indicators to strengthen the interpretation of the metabolic consequences associated with inadequate folate intake and the reversal of these changes with more optimal folate intakes. These data show that this assessment tool can be used in metabolic research protocols in conjunction with other indicators of folate status to characterize the adequacy of folate intake. 

JMW was a graduate student who performed the urinary folate catabolite assay and assisted with manuscript preparation. KH-G assisted with the processing and laboratory analysis of blood and urine samples, assisted with data management, and managed daily laboratory operations. DWT provided statistical advice and performed the data analysis. JFG provided technical advice for the urinary folate catabolite assay and contributed to manuscript preparation. GPAK and LBB, co-principal investigators for this project, secured and administered grant funding; developed the research design, feeding protocol, and folate-controlled diet; assisted with specimen processing; coordinated and managed all aspects of the experimental protocol; and prepared the manuscript for publication. None of the authors had any conflict of interest.

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