Kinetic Model of Folate Metabolism in Nonpregnant Women Consuming [\(^2\text{H}_2\)]Folic Acid: Isotopic Labeling of Urinary Folate and the Catabolite para-Acetamidobenzoylglutamate Indicates Slow, Intake-Dependent, Turnover of Folate Pools\(^1,2\)

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ABSTRACT In a 10-wk study of folate metabolism in nonpregnant women (21–27 y, \(n=5–6\) per group), subjects were fed a diet containing \(\sim 68\) nmol/d (30 \(\mu\)g/d) folate from food. The remainder of the ingested folate was provided as folic acid in apple juice (as nonlabeled during wk 1–2, as [\(^2\text{H}_2\)]folic acid during wk 3–10) to yield a constant intake of 454, 680 or 907 nmol/d (200, 300 or 400 \(\mu\)g/d). Isotopic enrichment of total urinary folate and the primary catabolite para-acetamidobenzoylglutamate (ApABG) was determined. Isotopic enrichment of ApABG served as an indicator of labeling of tissue folates. A kinetic model consisting of fast- and slow-turnover nonsaturable pools and a saturable slow-turnover pool, with provisions for urinary and fecal excretion, catabolism and enterohepatic circulation, yielded a close fit to the data. Mean residence times for total body folate were 212, 169 and 124 d for folate intakes of 454, 680, and 907 nmol/d, respectively. The model predicted that variation in folate intake over this range had little effect on the mass of the large saturable folate pool; however, the fast-turnover nonsaturable pools increased in proportion to folate intake, whereas the slow nonsaturable pool also tended to increase. This model will aid in evaluation of folate turnover and in predicting kinetic consequences of physiologic conditions associated with altered folate requirements. J. Nutr. 128: 1896–1906, 1998.

KEY WORDS: folate • kinetics • modeling • requirements • stable isotopes • humans

Adequate nutritional status for folate depends on a long-term intake to provide concentrations of the various tetrahydrofolate (\(\text{H}_4\text{folate}\))\(^4\) coenzymes in tissues sufficient to maintain optimal metabolic function. Concentrations of folate coenzymes in vivo depend largely on the quantity and bioavailability of ingested folate and the rate of their loss by urinary and fecal routes and through catabolism, although these relationships have not been fully defined. A better understanding of these relationships among the overall rate of folate turnover, mass of various in vivo pools, intake and bioavailability may aid in defining the nutritional requirement for this vitamin more precisely.

Understanding of the public health importance of adequate folate nutrition is rapidly expanding. Folate nutrition is intimately linked to cellular replication and homeostasis through the function of folates in nucleic acid synthesis, methionine regeneration, and in the shuttling of one-carbon units that function in many aspects of metabolism and regulation. Inadequate folate nutrition is associated with increased risk of neural tube defects (Scott et al. 1995), certain forms of cancer (Mason 1995), and many forms of vascular disease (Boushey et al. 1995, Morrison et al. 1996, Rimm et al. 1998). The metabolic effects of folate deficiency involved in these disease processes may include elevation in plasma homocysteine concentration (Selhub et al. 1993), impaired nucleic acid synthesis (Wagner 1995), reduced methylation of regulatory elements of certain genes (Mason 1995), and increased DNA fragmentation due to misincorporation of uracil (Blount et al. 1997, Pogribny et al. 1997). The long-term goal in understanding of folate requirements should involve defining intakes that minimize such deleterious processes and optimize folate-dependent processes in metabolism and cellular development. Investigation of the in vivo kinetics of folate provides an integrated view of the relationships among rates of intake, turnover, masses of in vivo folate pools and relative significance of excretory processes. Such information will strengthen our understanding of how changes in folate intake influence the quantity of folate available for metabolic processes and will aid in defining the nutritional requirement for folate more fully.

The study of in vivo kinetics and the development of mathematical models in human subjects provide information relevant to our understanding of nutrient requirements that
does not depend on interpretation of results from animal models, may yield greater insight into the physiology of nutrient processing and metabolism, and allows simulation and prediction of the effects of altered nutritional or physiologic conditions. As reviewed recently (Gregory and Scott 1996), the in vivo kinetics of folate metabolism and turnover have been examined in animals and humans. Studies of short-term kinetics yield useful information regarding plasma concentrations after various folate doses (Anderson et al. 1992, Bunni et al. 1989, Loew et al. 1987, Menke et al. 1993, Priest et al. 1991, Rogers et al. 1997), but they are not suitable for development of mathematical models of long-term whole-body folate kinetics. Kinetic investigations of folate turnover in animals have provided evidence of at least two identifiable pools, demonstrated by direct analysis of tissues (Bhandari and Gregory 1992, Lakshmaiah and Banji 1981, Murphy and Scott 1979, Scott and Gregory 1996, Tamura and Halsted 1983). Several previous studies have examined folate kinetics in humans, although none has provided a full kinetic model nor has the influence of nutritional status been investigated. Krumdieck et al. (1978) administered radiolabeled folic acid to a single female subject and observed substantial catabolism and fecal excretion in folate turnover with an apparent half-life of $\pm 100 \text{d}$ for the primary folate pool. Fecal excretion of labeled folate or catabolites was found to be an important excretory process, and the presence of labeled pterins in urine indicated that cleavage of the folate molecule was a catabolic process. Cleavage of the 9C-10N bond of the folate molecule is the only known mechanism of folate catabolism (Murphy et al. 1976, Murphy and Scott 1979); the major catabolic product in urine is the para-acetamido derivative of para-aminobenzoylglutamate (pABG) (McPartlin et al. 1992, Murphy et al. 1976, Murphy and Scott 1979). Additional kinetic studies in human subjects have suggested that folate turnover is accelerated by high intakes ($\pm 2$–5 mg/ d; Russell et al. 1983, Von der Porten et al. 1992), although the dose dependence of turnover rate at lower intakes has not been determined.

This research group has reported a preliminary kinetic model of whole-body folate turnover in human subjects on the basis of chronic administration of deuterium-labeled folic acid (Stites et al. 1997). The model consisted of a small fast-turnover pool in equilibrium with a large slow-turnover pool, with a provision for urinary excretion of intact folate and for other losses, i.e., fecal and catabolic. On the basis of measurement of isotopic enrichment of urinary folate, it was predicted that folate turnover would be very slow, as reflected by an apparent fractional catabolic rate of $0.008 \pm 0.001 \text{d}^{-1}$ ($\pm_{\text{SEM}}$, $n = 4$) for estimated total folate intakes of $649$–$1324$ nmol/d ($286$–$584 \text{mg} \mu \text{mol}^{-1}$). Several limitations of this modeling approach were as follows: 1) modeling was imprecise because calculations were based on urinary folate excretion, which comprised only $1$–$2\%$ of folate turnover; 2) the relative significance of fecal excretion and catabolism could not be determined; 3) folate intake was not controlled; and 4) predicted masses of in vivo pools were directly proportional to folate intake. However, this study provided new quantitative information regarding long-term folate metabolism and demonstrated the feasibility of long-term kinetic modeling of folate metabolism using chronic administration of deuterium-labeled folic acid.

We have conducted a controlled dietary study in which nonpregnant women were fed diets that provided intakes of $454$, $680$ or $907$ nmol/d ($200$, $300$ or $400 \text{mg} \mu \text{mol}^{-1}$) for $10 \text{wk}$. Diets were designed to provide only $68$ nmol/d ($30 \text{mg} \mu \text{mol}^{-1}$) of folate from food sources, with the remainder given as synthetic folic acid in apple juice. During wk 1–2, the synthetic folic acid was not isotopically labeled, whereas during wk 3–10, a portion of this folic acid was deuterium-labeled. Results of this study regarding relationships of dietary intake and serum and erythrocyte folate concentration, urinary folate excretion, and plasma homocysteine concentration have been reported previously (O'Keefe et al. 1995). We report here additional results of this study including isotopic excretion as urinary folate and the primary catabolite, para-acetamidobenzoylglutamate (pABG), as a function of folate intake, along with the development of an expanded kinetic model of folate metabolism. The data derived from this study and the resulting model will provide an initial quantitative picture of whole-body folate metabolism in adequately nourished young women and will serve as a basis for additional evaluation of conditions associated with changes in folate requirements.

**SUBJECTS AND METHODS**

**Overview of protocol.** The details of the protocol and diet composition have been reported previously (O'Keefe et al. 1995). Nonpregnant female subjects ($n = 18$, age $21$–$27$ y, weight $47$–$67$ kg) had normal blood chemistry and were in good health as reflected by a medical history and examination by a physician. This study was approved by the University of Florida Institutional Review Board. Informed consent was obtained from each subject. Subjects were randomly assigned to three treatment groups, $n = 6$ per group. One subject withdrew for personal reasons midway through the study. The protocol was conducted on an out-patient basis at the University of Florida Clinical Research Center. Consumption of meals and supplements was supervised by research personnel, and compliance was encouraged through daily interaction with the subjects. Adequacy of vitamin and mineral intake was ensured by administration of folic-free vitamin supplements (Fos Free, Mission Pharm, San Antonio, TX), a mineral supplement (Solgar Chelated Solamins Multiminerals, Solgar Vit, Lynbrook, NY), a potassium supplement (K-DUR 10, Key Pharm, Kenilworth, NJ) and a calcium supplement (Albertsons, Boise, NJ). Intake of dietary energy, protein, and fat and of supplemental vitamins and minerals was reported previously (O'Keefe et al. 1995).

Blood and urine collections were conducted at designated intervals throughout the study. All kinetic analysis was based on urinary folate and pABG excretion. For this purpose, 24-h urine collections were made directly into acid-washed plastic 2-L bottles containing $5$ g of dry sodium ascorbate. Each 24-h collection was begun after the first morning void and included the first void of the following morning. These bottles were kept refrigerated (2–4°C) during the collection period. Immediately after the 24-h collection was completed, total volume was measured, $50$ mL portions were transferred to polyethylene vials, saturated with nitrogen gas and stored at $-30^\circ$C until analyzed. All blood collection and analysis procedures were reported in the previous paper (O'Keefe et al. 1995). Three diet composites were collected for measurement of total folate, as described and reported previously (O'Keefe et al. 1995).

**Folic acid sources administered.** Nonlabeled folic acid supplements were prepared from commercially available folic acid (Sigma Chemical, St. Louis, MO), whereas the [3,5,7$^2$H$_2$]folic acid (FH$_2$folic acid) used as a stable-isotopic tracer was synthesized in this laboratory (Pfeiffer et al. 1997). Each was analyzed to verify purity and identity by HPLC, proton nuclear magnetic resonance and gas chromatography-mass spectrometry (GCMS) before use (Gregory 1990). Solutions of each were prepared in $0.1 \text{mol} \text{L}^{-1}$ PBS (pH 7.0) and the concentration determined spectrophotometrically using the molar absorptivity coefficient of $27,600 \text{L} \text{mol}^{-1} \text{cm}^{-1}$ (Blakley 1969). Appropriate volumes of each solution were dispensed into commercial pasteurized apple juice and stored as 45-mL portions in 50-mL conical centrifuge tubes, saturated with nitrogen gas and stored at $-30^\circ$C until used. During d 1–14, the supplemental folic acid consisted of nonlabeled folic acid in apple juice (45-mL portions given at morning and evening meals). During d 15–70, the supplemental folic acid consisted of an equimolar blend of nonlabeled ([FH]) and [FH$_2$]folic acid in apple juice (45-mL portions given at morning and evening meals).
evening meals). The concentration of these forms of folic acid in the apple juice was confirmed by HPLC (Gregory and Toth 1988), and stability was verified by analysis at several times throughout the study. A summary of the folate intake of subjects in each group is shown in Table 1.

The final phase of this study was to determine whether the urinary excretion of a single bolus dose of a labeled folate could be used as a functional indicator of folate status. For this purpose, the following single-day protocol was conducted immediately after the study described in this paper.

After completion of the last urine collection on d 70, a single dose of [glutamate-\(^{2H_4}\)]folic acid ([\(^{1H_4}\)folic acid] [1134 nmol (500 \(\mu g\)] Gregory and Toth 1988) was administered to each subject; then each subject collected urine for 24 h. The results of this short-term study are reported in a separate communication (Gregory et al. 1998).

Analytical methods. Documentation of folate nutritional status. As described previously, serum, erythrocyte and urinary folate concentrations were determined by microbiological assay with Lactobacillus casei (Tamura 1990). Total plasma homocysteine concentrations were determined by a fluorometric HPLC procedure (Vester and Rasmussen 1991).

Determination of urinary folate by HPLC and preparation of urinary folate for GCMS analysis. Urinary folate concentration was determined by HPLC after affinity chromatography (Gregory and Toth 1988). This method is based on isolation and purification of urinary folate using columns packed with Affigel 10 (BioRad Laboratories, Hercules, CA) coupled to bovine milk folate-binding protein. Recovery of 5-methyl-H\(^4\)folate and folic acid added to urine was typically 95%. Care was taken to maintain the quantity of total folate applied to the affinity column at <30% of column capacity to ensure high recovery of all folates. The 5-mL fraction containing folate eluted from this column was divided as follows: 1 mL was used for HPLC analysis (Gregory and Toth 1988, Stites et al. 1997); the remainder prepared for GCMS analysis involves intentional cleavage of the 9C-10N bond, isolation of the resulting \(p\)ABG by HPLC and derivatization with combined trifluoroacetic ester.

A summary of the folate intake of subjects in each group is shown in Table 1.

<table>
<thead>
<tr>
<th>Total folate intake</th>
<th>Stage of protocol</th>
<th>Folate from foods</th>
<th>Total synthetic folic acid</th>
<th>Nonlabeled folic acid</th>
<th>([3'5'-\text{\textsuperscript{2}}\text{H_4}]\text{Folic acid})</th>
<th>Isotopic enrichment of total ingested folate</th>
</tr>
</thead>
<tbody>
<tr>
<td>nmol/d</td>
<td>d</td>
<td>nmol/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>454 (200 (\mu g/d))</td>
<td>1–14</td>
<td>68</td>
<td>386</td>
<td>386</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>680 (300 (\mu g/d))</td>
<td>15–70</td>
<td>68</td>
<td>368</td>
<td>193</td>
<td>193</td>
<td>0.425</td>
</tr>
<tr>
<td>907 (400 (\mu g/d))</td>
<td>1–14</td>
<td>68</td>
<td>612</td>
<td>612</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>15–70</td>
<td>68</td>
<td>306</td>
<td>306</td>
<td>0</td>
<td>0.450</td>
</tr>
<tr>
<td></td>
<td>15–70</td>
<td>68</td>
<td>839</td>
<td>839</td>
<td>0</td>
<td>0.463</td>
</tr>
</tbody>
</table>

1. Folate sources included endogenous food folate, nonlabeled folic acid and [3'5'-\text{\textsuperscript{2}}\text{H_4}]folic acid.
mine moiety was removed by zinc-HCl treatment, continuing as described by McPartlin et al. (1992). This entire solution was subjected to preparative HPLC, and the pABG peak collected and evaporated to dryness (Pfeiffer and Gregory 1997). Derivatization for GCMS analysis was then performed as described above (Gregory and Toth 1988).

Gas chromatography-mass spectrometry analysis of pABG. GCMS analysis of derivatized pABG (derived from both urinary folate and urinary ApABG) was performed as previously described in electron-capture negative ionization mode with selected-ion monitoring at mass-to-charge ratios (m/z) 426 and 428 (Gregory and Toth 1988). All analyses were performed using a Hewlett-Packard Model 5890 GCMS system (Palo Alto, CA) with methane as reagent gas. Working standard response curves were prepared by using known mixtures of labeled [2H2] and nonlabeled pABG (prepared from known mixtures of [1H2] and nonlabeled folic acid) to determine the relation between ratios of observed peak areas in GCMS analysis by selected-ion monitoring and the actual molar ratios of labeled and nonlabeled folates. All standard mixtures and samples were analyzed in duplicate or triplicate, and ratios of labeled and nonlabeled forms of pABG were determined using simultaneous equations that corrected for the natural abundance of isotopomers.

Kinetic modeling. All modeling was conducted using the compartmental analysis module of SAAM II software, version 1.1 (SAAM Institute, University of Washington, Seattle, WA; Foster et al. 1994) on a personal computer. The model developed in this study was based on that reported previously (Stites et al. 1997), with changes described below to increase its physiologic accuracy. The previous model consisted of a fast-turnover (Pool 1) and a slow-turnover pool (Pool 6), the latter presumably comprised of folates associated with tissues; both of these are nonsaturable compartments. Ingested folate (Pool 5) entered Pool 1, with assumed 67% absorption. The assumed 67% bioavailability was based on the reported low bioavailability of many sources of food folate (Sauberlich et al. 1987) and the observed ~85% absorption of folic acid (dissolved in apple juice) when consumed with a light meal (Pfeiffer et al. 1997). This preliminary model had provisions for losses as urinary folate and via other routes (catabolic and fecal). The expanded model devised for this study (Fig. 2) included these pools plus the following additional characteristics: 1) A third saturable pool (Pool 4) was included that represented the major slow-turnover folate compartment. 2) Provisions were made for urinary excretion of intact folates from Pools 1 and 6. 3) Additional provisions were introduced for catabolic losses from tissue folate pools (Pools 4 and 6). 4) Losses of folate via fecal excretion also were included from both Pools 4 and 6. 5) Secretion of folate from Pool 6 into an intestinal compartment (Pool 7) was included to represent digestive secretions such as bile and pancreatic juice. After a 0.5-d delay (compartment 8), this secreted folate entered Pool 9 from which a large fraction is reabsorbed back to Pool 1, whereas the fraction not reabsorbed from Pool 9 undergoes fecal excretion. 6) Provision also was made for incomplete bioavailability of dietary folate (again assumed to be 67% overall) by adjusting the fraction of ingested folate entering Pool 5 that underwent transfer to Pool 1. As in previous modeling, compartment 2 and compartment 3 had no anatomical or physiologic equivalent but were simply a sink for totaling catabolic losses (as ApABG) and urinary excretion of folate, respectively.

Sources of folate intake were designated in SAAM II modeling as “endogenous input” (total nonlabeled folate, i.e., food folate and nonlabeled folic acid), and “exogenous input” ([2H2]folic acid), at levels shown in Table 1. The mass of Pool 4, the large, saturable, tissue folate pool, was defined by a Michaelis-Menten expression, \( \frac{J_{\text{max}} \times \text{mass}(6))}{\text{mass}(4) + \text{mass}(6)} \), with subtraction of first-order-based outflow to Pools 2, 6 and 10. In this expression, \( J_{\text{max}} \) was estimated to be the total binding capacity of Pool 4 and \( K_d \) was an empirical constant representing the overall dependence of saturable, processes and saturable binding on the mass of free folate in tissues.

In this model developed with SAAM II, the exchange rate constants \( \{k(i, j)\} \) represented the fraction of folate in compartment \( i \) transferred to compartment \( j \) per unit time (d). The mass transferred from compartment \( j \) to compartment \( i \) per unit time was termed the flux \( (J, J) \), which represents mass \( (J) \times k(i, j) \).

The following assumptions were made in modeling on the basis of known aspects of folate metabolism: 1) The mass of the rapid-turnover pool (Pool 1) is much smaller than that of the combined main tissue folate pools (Pools 4 and 6). 2) Although the total mass of body folate has not been determined directly, we assumed on the basis of folate in human liver that total body folate mass would not exceed ~90 nmol (~40 mg expressed as monoglutamyl folate equivalents). 3) Mean concentration of bile folate was estimated to be ~90 nmol/L (~40 ng/mL; Lavoie and Cooper 1974), with a typical bile flow of 600–700 mL/d (Guyton 1971) and mean total biliary folate of

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**FIGURE 1** Typical chromatogram from fluorometric HPLC determination of total para-aminobenzoylglutamate (pABG) in urine. This analysis measures the sum of free pABG and its acetylated derivative acetamidobenzoylglutamate (ApABG).

**FIGURE 2** Kinetic model of folate metabolism in humans. Labels: Pool 5, GIT = gastrointestinal tract (into which all folate initially enters); Pool 1, Fast = rapid-turnover pool; Pool 6, Slow-F = slow-turnover free folate pool; Pool 4, Slow-B = slow-turnover bound folate pool; Pool 2, Uapabg = urinary para-acyetamidobenzoylglutamate (ApABG); Pool 3, Ufolute = urinary folate; Pool 7, SI = small intestinal pool into which biliary folate enters, followed by a 0.5-d delay (Pool 8); Pool 9, SI = small intestinal pool from which biliary folate is reabsorbed to Pool 1; Pool 10, Fecal = fecal folate derived from endogenous sources (e.g., digestive secretions and sloughed mucosal cells). Compartment 8 constitutes a delay affecting the rate of folate passage through enterohepatic circulation.
in Table 2. Significant differences were observed in serum folate and plasma homocysteine concentrations and urinary folate excretion (P < 0.05). Essentially all urinary folate was 5-methyl-H$_4$folate in HPLC analysis, with little or no unchanged folic acid regardless of folate intake.

No difference was seen among dietary groups with respect to total pABG excretion. Mean values for total urinary pABG excretion for intakes of 454, 680 and 907 nmol/d were 341 ± 75, 298 ± 41 and 342 ± 63 nmol/d, respectively (means ± SEM; four analyses per subject of samples collected over 4 wk midway through isotopic administration period). These results indicate that total pABG excretion greatly exceeded excretion of intact urinary folate and that the fraction of intake that it comprised varied inversely with the intake level. When acid hydrolysis was omitted in randomly selected samples, the pABG peak was reduced to <20% of that seen when using the acid hydrolysis reaction. Thus, pABG constituted >80% of the total catabolite excretion, consistent with data reported by McPartlin et al. (1992 and 1993) and Caudill et al. (1998).

This suggests that mean excretion of pABG was ~260 nmol/d [i.e., 80% of the mean total pABG excretion (327 nmol/d)]. This value is consistent with previous reports that pABG excretion was much greater than folate excretion.

**Isotopic enrichment of urinary folate and p-acetamidobenzoylglutamate.** Isotopic enrichment of urinary folate and urinary pABG increased gradually throughout the study. Maximum values were observed in the 907 nmol/d intake group and approached isotopic enrichments of 0.3. Relative to the calculated isotopic enrichment of total ingested folate of 0.425–0.465 ([H$_4$]folic acid/total ingested folate; Table 1), these results indicate that isotopic equilibrium of body folate pools was not reached within the 8-wk period of [H$_4$]folic acid administration.

The patterns of labeling for all groups were qualitatively similar at each folate intake. The major difference observed between groups was a greater initial rise in enrichment at the high intake.

**Kinetic modeling.** The model shown in Figure 2 provided good fit to the isotopic enrichment data for all subjects (for example, Fig. 3). Analogous modeling was then conducted after transformation of the data from isotopic enrichment to excretion (nmol/d) of urinary [H$_4$]folate and [H$_4$]pABG to allow evaluation of the relative extent of isotopic excretion by each of the primary routes (i.e., urinary [H$_4$]folate, urinary [H$_4$]pABG and apparent fecal excretion).

Because direct measurement of urinary pABG concentration was not performed in this study, we employed an assumed
ApABG excretion of 220 nmol/d for all subjects for the calculation of urinary [2H2]ApABG excretion from isotopic enrichment. This assumed value was chosen as intermediate between the estimated 260 nmol/d ApABG excretion in this study and values of 95 nmol/d (Caudill et al. 1998) and ~160 nmol/d (McPartlin et al. 1993) for ApABG excretion in nonpregnant women. Urinary excretion of [2H2]folate was calculated from measured urinary folate excretion and isotopic enrichment values. Application of the model to these excretion data yielded good fit. Representative results for each level of folate intake are shown in Figure 4.

A summary of the model-derived estimates of rate constants, overall fractional catabolic rates and overall mean residence times for each level of folate intake is presented in Table 3, and estimates of masses are shown in Table 4. The estimated mass of total body folate in this model was controlled primarily by the capacity and binding constant of the saturable pool. Several observations made during modeling merit comment. Urinary folate data could be accurately fit only when including output of urinary folate from both Pools 1 and 6 in the model. Similarly, urinary ApABG data could be accurately fit only when output was from both Pools 4 and 6. This model is fit to data for urinary excretion of [2H2]folate and [2H2]ApABG; thus, losses by fecal and/or other routes are estimated. A steady state is attained only when rate constants for fecal excretion are in the range shown in Table 3, which provides sufficient flux via the fecal route to keep the model in balance. With respect to overall folate turnover, as reflected by fractional catabolic rates, the main characteristic found in modeling was very slow turnover of whole-body folate. Model-predicted mean residence times were >100 d for all subjects and were >200 d for most subjects at the lowest folate intake. Finally, estimated masses of Pool 1, the rapid turnover pool, ranged from ~0.6 to 1.2 nmol for all subjects in this study. Dividing these mass(1) values by an assumed 3-L plasma volume yields an apparent concentration greater than that of plasma folate. This observation suggests that rapidly exchanging folates in certain tissues contribute to the rapid turnover folate pool in addition to plasma folate, although it also is possible that the model overestimates the mass of this pool.

The total folate intake of treatment groups was found to influence several kinetic parameters (Table 3). The rate constants for secretion of urinary folate, k(3,1) and k(3,6), at the highest folate intake were significantly greater than those of the lower two intake levels (P <0.05). A trend (P = 0.122) was observed for an effect of folate intake on the rate constant for catabolism from Pool 6 [k(2,6)]. Rate constants for fecal excretion of folate from Pool 6, k(10,6), were significantly greater at the higher two levels of folate intake. Whole-body fractional catabolic rates increased with increasing folate intake, with significant differences between values at each intake level (P < 0.05). This effect corresponded to large differences in mean residence times for whole-body folate (i.e., means of 212, 169 and 124 d for intakes of 454, 680 and 907 nmol/d, respectively). With respect to predicted masses (Table 4), a significant effect of folate intake was observed only for Pool 1, the small, rapid-turnover pool. However, trends that did not achieve significance that related folate intake with the mass of Pool 6 (P = 0.119) and total mass (P = 0.102) were found.

**FIGURE 3** Isotopic enrichment of urinary folate and urinary para-acetamidobenzoylglutamate (ApABG) excreted by nonpregnant women consuming [2H2]folic acid during chronic total folate intake of 454, 680 or 907 nmol/d. The solid and dashed lines are model predictions for isotopic enrichment of urinary folate and ApABG, respectively.
The ratio of the mass of Pool 6 to the combined masses of Pools 4 and 6 was also evaluated to determine the fraction of tissue folate in "free" (nonbound) form. The model predicted that 30 – 40% of folate in tissue pools was in free form. This fraction tended to increase \( P < 0.119 \) as a function of folate intake (Table 4). Because of the small number of subjects in each group \( n = 5 – 6 \), which yielded less than optimal statistical power, these trends are reported to convey information regarding the apparent response of the model.

**Model-based simulation studies.** The kinetic model developed here was used in several simulations to obtain preliminary predictions of the effects of several altered physiologic or nutritional conditions (Fig. 5). Simulations of higher and lower levels of folate intake showed that Pools 1 and 6 exhibited the greatest response. The saturable Pool 4 exhibited a slight reduction with decreasing folate intake and a slight increase with higher intakes.

### DISCUSSION

The kinetic model described here represents an extension of previous kinetic analyses of human folate metabolism because of the greater physiologic relevance of this model to

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**Figure 4** Excretion (nmol/d) of urinary \[^{2}H\text{2}\]folate and \[^{2}H\text{2}\]para-acetamidobenzoylglutamate (\[^{2}H\text{2}\]ApABG) by nonpregnant women consuming \[^{2}H\text{2}\]folic acid during chronic total folate intake of 454, 580 or 907 nmol/d. The solid and dashed lines are model predictions for urinary excretion of \[^{2}H\text{2}\]folate and \[^{2}H\text{2}\]ApABG, respectively.
FOLATE KINETICS IN NONPREGNANT WOMEN

Table 4

Model-derived estimates of mass of folate pools and total-body folate for nonpregnant women consuming total folate intakes of 454, 680 or 907 nmol/d

<table>
<thead>
<tr>
<th>Folate intake</th>
<th>454 nmol/d</th>
<th>680 nmol/d</th>
<th>907 nmol/d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 6)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>Mass(1), μmol</td>
<td>0.611 ± 0.003a</td>
<td>1.02 ± 0.06b</td>
<td>1.19 ± 0.003c</td>
</tr>
<tr>
<td>Mass(4), μmol</td>
<td>43.5 ± 0.13</td>
<td>43.5 ± 0.17</td>
<td>43.7 ± 0.07</td>
</tr>
<tr>
<td>Mass(6), μmol*</td>
<td>20.4 ± 2.2</td>
<td>27.0 ± 3.4</td>
<td>28.1 ± 2.3</td>
</tr>
<tr>
<td>Total mass, μmol*</td>
<td>64.5 ± 2.3</td>
<td>71.5 ± 3.6</td>
<td>73.0 ± 2.4</td>
</tr>
</tbody>
</table>

Parsimony is constrained with the assumptions that the mass(4) and mass(6) pools are independent of the mass(1) pool, and that the mass(6) pool is independent of the mass(4) pool.

1 Values are means ± SEM. Total mass = mass(1) + mass(4) + mass(6).
2 For mass(1) and mass(4), values were logarithmically transformed before ANOVA to improve normality and compensate for unequal variance. Within a row, values followed by a different superscript letter are significantly different, P < 0.05. For parameters marked with an asterisk (*), there was a nonsignificant trend (P = 0.119, P = 0.102 and P = 0.119) for ANOVA of mass(6), total mass, and the mass(6)/[mass(4) + mass(6)] ratio, respectively.

In designing this study, it was originally intended to conduct modeling on the basis of urinary excretion of labeled total pABG; thus determination of ApABG concentration in urine by the method of McPartlin et al. (1992) was not conducted. Once HPLC and GCMS analyses were complete and modeling was in progress, the advantage of kinetic calculations based on excretion of [H2]ApABG became apparent, but samples were no longer available for direct determination of urinary ApABG concentration. Conclusions of this study are reasonable on the basis of the assumed value for ApABG excretion. In the worst case, the assumed value is an overestimate of ApABG excretion, which would cause estimated rates of catabolism to be comparably underestimated. If that were the case, then fecal losses from tissue pools (Pools 4 and 6), which are already large (consistent with Krumdieck et al. 1978), would be even greater than currently seen in this model.

The inclusion of a saturable pool as the major tissue folate compartment contributes to the relevance of the model. In rat liver, ~60% of cytosolic folate and 20% of mitochondrial folate are specifically bound to proteins (Zamierowski and Wagner 1977). These observations compare favorably to the model-based predictions regarding the fraction of free folate in tissue pools of this study (Table 4). The model-based prediction that the fraction of free folate in tissues increases with increasing folate intake is also consistent with the observations of Zamierowski and Wagner (1977) that free folate is depleted to a greater extent than protein-bound folate during folate deficiency. Previous studies in rats have shown that tissue folate mass and/or concentration increases in a nonlinear fashion with increasing folate intake ranging from deficient to optimal levels (Clifford et al. 1990, Keagy 1982). These observations also support the need for a major saturable pool in modeling.

Although three in vivo pools are kinetically identifiable from our data, pools involving enterohepatic circulation have been included for physiologic relevance (Steinberg 1984), not kinetic identifiability at this stage. The delay included in enterohepatic circulation has little effect on the model. Very rapid aspects of folate absorption and distribution, as seen in a recent stable isotopic study of plasma kinetics (Rogers et al. 1997), have not been included because of the 24-h sampling times used in this protocol. No effort has been made to account for the metabolic interconversion and function of folates. Several modeling studies that are based on kinetic evaluation of folate metabolism and the effects of antifolates in cell culture or cell-free systems have been reported (Jackson and Harrap 1973, Seither et al. 1989, White 1979). However, the present modeling is directed primarily at examining whole-body turnover, and incorporation of such metabolic interconversions of folate coenzymes into the current model is not justified or necessary. The current model is a reasonable approximation of whole-body folate turnover and represents a starting point upon which to base additional modeling and simulation to examine other physiologic conditions (e.g., pregnancy).

Figure 5

Model-derived prediction of the relationship between mass of folate pools and folate intake at steady state for human subjects. Data points for total folate intakes of 454 and 907 nmol/d are model-derived estimates of folate pools in nonpregnant women in this study. Modeling simulations for intakes < 454 nmol/d and > 907 nmol/d were based on rate constants determined from models of those subjects.
The total body mass of folate in adult humans has not been precisely determined, as discussed previously (Stites et al. 1977). Herbert (1987) estimated a total body folate mass of \(7.5 \pm 2.5\) mg (17 \(\pm\) 5.7 \(\mu\)mol). Hoppner and Lampi (1980) analyzed 560 human livers and reported a mean of 8.0 \(\pm\) 2.8 \(\mu\)g (18.1 \(\pm\) 6.3 nmol/g), and a similar range was reported by Whitehead (1973). Assuming that liver folate comprises half of total body folate and that human liver mass is 1400 g, then the last-mentioned two studies would suggest that total body folate would be approximately 22 mg or 50.8 \(\mu\)mol. The model provides slightly higher estimates (Table 4) for the nutritionally relevant folate intakes of this study. Even higher values reported previously (Stites et al. 1997) are probably overestimates because the model did not include a saturable pool.

As stated above, a long-range goal of this modeling effort is to provide kinetically based information regarding the human requirement for folate. Attempts have been made to derive requirements for other vitamins on the basis of kinetic analysis, e.g., ascorbic acid, although determining a requirement on the basis of kinetic data remains problematic (Shane 1997, Young 1996). Several observations from this study, however, may provide information regarding optimal intake. Rate constants for urinary excretion of folate \(k(3,1)\) and \(k(3,6)\) increased substantially between intakes of 680 and 907 nmol/d (300 and 400 \(\mu\)g/d), consistent with elevated urinary excretion of labeled and nonlabeled folate at the 907 nmol/d intake level (Table 2). This may have been due to saturation of renal reabsorption mechanisms and possibly increased secretion of free renal folate as predicted by the increase in \(k(3,6)\).

The existence of renal tubular secretion of folate has been reported previously and characterized recently (Morshed et al. 1997). These kinetic and analytical findings indicate greater loss of urinary folate between intakes of 680 and 907 nmol/d. A second line of inference involves the observation that plasma homocysteine was significantly greater in the 454 nmol/d intake group, with several subjects exhibiting concentrations of plasma homocysteine >15 nmol/L, which suggests marginal deficiency at that level of intake (O’Keefe et al. 1995). The model predicts a trend toward increases in the mass of free folate (Pool 6) with increasing folate intake; the mass of Pool 6 was significantly correlated with plasma homocysteine concentration, and increase in plasma homocysteine at the 454 nmol/d intake (similar to the observed \([2H_2]\)ApABG excretion (Fig. 4) as well as steady-state estimates of catabolic flux [calculated as the respective rate constants, \(k(4,2)\) and \(k(6,2)\), for ApABG formation] and \(x\) mass of Pools 4 and 6] do indicate that ApABG excretion is influenced by folate intake. Similarly, in a study of supplementation with higher levels of folic acid in addition to a low folate diet (120 \(\mu\)g dietary folate plus folic acid to yield either 450 or 850 \(\mu\)g/d), we have shown that the daily excretion of ApABG and the minor nonacylated form pABG does increase significantly with increasing folate intake (Caudill et al. 1998).

Fecal excretion accounts for a substantial proportion of human folate turnover (Krumdieck et al. 1978). The model predicts that fecal losses from tissues (Pool 6) increase with increasing folate intake (i.e., not simply unabsorbed dietary folate). This conclusion is based on the substantially (although not significantly) greater values of \(k(10,6)\) at the higher two folate intakes. Unfortunately, direct determination and interpretation of fecal \([H_2]\)folate, labeled catabolic products, or even total deuterium are not possible because of the confounding bacterial synthesis of folate and the high natural abundance of deuterium. Thus, one cannot calculate a requirement on the basis of isotopic balance. Also, one cannot use these kinetic results to estimate a minimally adequate pool mass or the quantity that must be replaced daily. Predictions of whole-body folate in this study indicated means of 64.5 \(\pm\) 2.3, 71.5 \(\pm\) 3.6 and 73.0 \(\pm\) 2.4 \(\mu\)mol for intakes of 454, 680 and 907 nmol/d, with fractional catabolic rates of 0.00474, 0.00607, and 0.00822/d, respectively. On the basis of the observations regarding homocysteine concentrations, one might infer that a

![Table 5](https://academic.oup.com/jn/article-abstract/128/11/1896/4722453)

Table 5: Evaluation of linear correlation between predicted masses of folate pools and criteria of folate nutritional status using Pearson product-moment correlation procedure

<table>
<thead>
<tr>
<th>Variable</th>
<th>Serum folate</th>
<th>Erythrocyte folate</th>
<th>Plasma homocysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass(1)</td>
<td>0.507 (0.0378)</td>
<td>0.405 (0.107)</td>
<td>-0.607 (0.000983)</td>
</tr>
<tr>
<td>Mass(4)</td>
<td>0.438 (0.0825)</td>
<td>0.322 (0.208)</td>
<td>-0.989 (0.000000)</td>
</tr>
<tr>
<td>Mass(6)</td>
<td>0.504 (0.0391)</td>
<td>0.150 (0.566)</td>
<td>-0.236 (0.361)</td>
</tr>
<tr>
<td>Mass(total)</td>
<td>0.570 (0.0169)</td>
<td>0.170 (0.513)</td>
<td>-0.250 (0.332)</td>
</tr>
</tbody>
</table>

1 Values shown are Pearson product-moment correlation coefficients, with \(P\)-values shown in parentheses. Coefficients with \(P < 0.05\) were considered to indicate a significant linear relationship. Positive correlation coefficients indicate that both variables increase together, whereas negative values indicate an inverse relationship. Correlation coefficients for associations among folate pools were: mass(1) vs. mass(4), \(r = 0.189 (P = 0.470)\); mass(4) vs. mass(6), \(r = 0.553 (P = 0.0213)\); mass(1) vs. mass(total), \(r = 0.570 (P = 0.00169)\); mass(4) vs. mass(total), \(r = 0.707 (P = 0.000152)\); mass(6) vs. mass(total), \(r = 0.999 (P < 0.0001)\).
predicted whole-body folate mass of ~70 μmol is adequate to maintain this aspect of folate-dependent one-carbon metabolism. Studies involving more precise modeling and the use of additional functional indicators of folate status are required to resolve such issues. In this model, the compartments that would correspond primarily to metabolically active folates in tissues (i.e., Pools 4 and 6) are comprised of a number of chemical forms of the vitamin that exhibit similar turnover kinetics. As stated previously, Pool 1 may also consist of some metabolically active folates in tissues. Within each of these pools, folate molecules may undergo metabolic interconversions and transport between cytosol and mitochondria or other organelles. On the basis of the kinetic data and model of this study, one cannot interpret precisely the function of various folates in these pools.

Mean residence times for whole-body folate were 212 ± 8, 169 ± 12 and 124 ± 7 d for intakes of 454, 680 and 907 nmol/d, respectively. These findings illustrate the difficulty in designing and interpreting folate nutritional studies as a result of the very long time required to fully achieve a new steady-state level in response to a dietary change or supplementation. The 10-wk feeding periods used in this study would not have allowed the subjects to fully attain new steady-state levels with respect to folate pool masses, although this protocol was sufficient for substantial differences to be observed. Assuming the kinetic rule-of-thumb that five times t\textsubscript{1/2} is required to achieve ~95% of the steady-state level (and t\textsubscript{1/2} of whole body folate was 0.693 × mean residence time), we predict that a study of ~500 d would be required to be fully assured that subjects had achieved constant body pools if using a low folate intake (~454 nmol/d). It is interesting to note that the mean residence time decreases with increasing folate intake, which suggests that newly absorbed folate molecules compete for binding sites in tissues and thus accelerate turnover.

In summary, the kinetic study and compartmental model reported here have extended our quantitative understanding of folate metabolism in humans. The kinetic data and model reported complement observations reported previously regarding nutritional status and folate intake (O’Keefe et al. 1995). Simulations based on this model permit a preliminary prediction of the effect of nutritional and physiologic conditions that are neither experimentally feasible nor practical. The results of this study give a baseline from which to compare additional kinetic studies to evaluate kinetic criteria of such conditions associated with altered folate requirements.

LITERATURE CITED


