Estimation of the 5-Methyltetrahydrofolate Apparent Volume of Distribution in Humans

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Abstract

The fractional absorption of a stable isotope-labeled folate dose can be estimated from the subsequent short-term temporal changes in the concentration of labeled L-5-methyltetrahydrofolate (L-5-methyl-THF) in plasma using mathematical modeling. However, the model is dependent on the use of an accurate value for the apparent volume of distribution of L-5-methyl-THF. Previous studies that estimated the apparent volume of distribution of L-5-methyl-THF used large (nonphysiological) doses of unlabeled folates that are not found to any great extent in the circulatory system. The current study estimates the apparent volume of distribution at steady state in 16 healthy humans aged 18–65 y after an i.v. dose (440 nmol) of a stable isotope-labeled version of the naturally circulating plasma folate, L-5-methyl-THF. Blood was collected from 2 min to 2 h postinjection and plasma assayed by specific and sensitive liquid chromatography-tandem MS. The apparent volume of distribution for L-5-methyl-THF was 32.0 ± 11.6 L (mean ± SD; 392 ± 110 mL/kg bodyweight). There was a positive association with volunteer body weight (r = 0.64; P = 0.010), which allowed a simple linear equation to be developed relating apparent volume of distribution to body weight. This has important implications for predicting apparent absorption of labeled folates in future bioavailability studies.

Introduction

Folates form a family of water-soluble B vitamins that have similar chemical structures to folic acid and are vital for methionine and nucleotide biosynthesis (1). An overview of the mechanism of folate absorption in humans can be found elsewhere (2). Low folate status has been associated with altered DNA methylation, which may affect gene expression (3), and uracil-induced genomic instability (4), which may result in increased cancer risk. Furthermore, low folate status is associated with elevated plasma homocysteine and increased risk of cardiovascular disease (5) and stroke (6) and has been linked to dementia and Alzheimer’s disease (7). A significant reduction in the incidence and occurrence of neural tube defects, such as spina bifida, has been demonstrated in women who increase periconceptional intake of supplemental folic acid. In countries where there are no current policies for mandatory folic acid fortification, there is a need to understand the absorption and bioavailability of food folate to address the question of whether optimal folate status can be achieved by food folate intake alone or whether supplementation of dietary intake is necessary.

Mathematical modeling has been employed to assist in the estimation of folate absorption or comparative absorption relative to a reference dose of folic acid. This approach was used in a human study where the fractional absorption of oral doses of stable isotope-labeled folates ([13C6]folic acid, (6S)-[13C6]5-formyltetrahydrofolic acid, and intrinsically-labeled [15N1–7] spinach) were estimated from the temporal changes in the labeled 5-methyltetrahydrofolic acid plasma concentration by the use of a single compartment model (8). However, the model is dependent on the use of an accurate apparent volume of distribution for L-5-methyl-THF in that single compartment. Apparent volume of distribution is a pharmacokinetic term normally defined as the volume in which the amount of an injected (or infused) drug (or substance) would need to be uniformly distributed to produce the observed plasma concentration (9). The apparent volume of distribution does not usually have an exact anatomical meaning, because its value can reflect...
binding to plasma proteins or extravascular tissues, which, respectively, ensure that the apparent volume is smaller or larger than the real volume of distribution (10).

Confusion also arises in the naming conventions given to the apparent volume of distribution and how they relate to single and multi-compartment systems. For a drug or substance that appears to distribute into more than one compartment, the sampled compartment’s volume of distribution is given the symbol V_c. This is also the initial apparent volume that the substance occupies in the entire body just after an i.v. bolus (at time 0). This is not the true apparent volume of distribution of the substance, because a finite time is required for it to distribute after being injected. It is therefore more appropriate to estimate the volume of distribution once some time has elapsed. One such measure is the apparent volume that the substance occupies during the terminal exponential phase of the (substance) plasma concentration vs. time graph. The theory behind this is that even a substance that appears to occupy many distinct compartments will eventually equilibrate within the system and be excreted as though it was in a single (lumped) system. This volume is given the symbol V_b. Although V_b is a more useful measure than V_c, the most widely used estimate of volume of distribution is the volume occupied by the substance at steady state, known as V_ss. This is the sum of the apparent volumes of the central and peripheral compartments in a multi-compartment system (10). Generally, in a multi-compartment system, the order of size for these various estimates is V_c < V_m < V_b. If a substance appears to occupy only a single compartment, all the apparent volumes are equivalent (i.e., V_c = V_m = V_b).

The model that was previously developed to estimate the apparent volume of distribution reflected the fact that it is a “lumped” or amalgamated multi-compartment system and its apparent volume of distribution therefore needs to be estimated by V_b or apparent volume of distribution, not V_c.

Previous work in estimating the apparent volume of distribution has recognized that even the short-term kinetics of folate absorption and elimination (8). This is known to be a simplification of the true kinetics for folates and the apparent volume of distribution used in that model needs to reflect the fact that it is a “lumped” or amalgamated multi-compartment system and its apparent volume of distribution therefore needs to be estimated by V_b or apparent volume of distribution, not V_c.

In the present study, apparent volume of distribution at steady state was estimated using a physiological i.v. dose (∼440 nmol) of a 13C stable isotope-labeled version of the naturally circulating plasma folate, 5-methyltetrahydrofolinic acid. Labeled [13C5]-5-methyltetrahydrofolic acid was assayed in blood plasma by a specific and sensitive LC-MS/MS method from 2 min to 2 h postinjection. Correlations of the apparent volume of distribution with other participant characteristics were explored with a view to establishing an equation to predict the apparent volume of distribution from easily measured anthropometric parameters for use in future modeling work.

Materials and Methods

Preparation of 13C5-labeled i.v. doses. The calcium salt of (6S)-[13C5]-5-methyltetrahydrofolic acid (calcium-L-mefolinate-13C5, labeled on the 5 carbons of the glutamate moiety) was purchased from Merck Eprova in 20-mg sealed vials. These were transported to the Ipswich Hospital Pharmacy Manufacturing Unit for preparation into single-use i.v. doses.

Each vial was reconstituted in 0.9% (wt/v) sodium chloride to a final volume of 100 mL (final concentration ∼0.38 mmol/L). The solution was filter-sterilized through a 0.2-µm sterile filter and 2.0-mL aliquots were aseptically transferred to sterile single-dose ampoules. Sterility and pyrogen testing were performed at the Ipswich Pharmacy Manufacturing Unit. The chemical identity and purity of the doses was confirmed using the LC-MS/MS method described later. Doses were quantified by spectrophotometric analysis of the second- and penultimate-filled vials. Absorbance of a 1 in 20 dilution of ampoule contents into 0.1 mol/L sodium phosphate buffer, pH 7.0, was read at 290 nm and the concentration (mmol/L) was calculated using a molar extinction coefficient of 31,700 (mol/L)−1 cm−1 (14). The shelf life of the prepared doses was 60 days at −20°C from the day of preparation and two batches of the i.v. dose were prepared over the course of the study.

Participants and study design. Following approval from the Institute of Food Research’s Human Research Governance Committee, the study was approved by the Norfolk Research Ethics Committee. Additional ethical permission was also given for the use of RBC folate concentration and MTHFR C677T genotyping data acquired from a concurrent study using the same volunteers. Healthy males and females aged 18–65 y were recruited from Norwich and the surrounding area. Written informed consent was obtained at screening, when volunteers were asked to complete a health questionnaire and data on height and weight were obtained to allow estimation of BMI. A urine sample was provided by the volunteer and dipstick-tested for pH, protein, glucose, ketones, urobilinogen, bilirubin (Bayer Multistix SG, Bayer Healthcare). A blood sample was taken from fasting participants for measurement of full blood count, urea and electrolytes, and glucose.

As part of a concurrent study, additional fasting blood samples were also provided for RBC folate analysis and MTHFR C677T genotyping. Vein status for the suitability of cannulation and i.v. injection was also assessed at screening. Suitable potential participants who passed the cannulation study screening stage were then invited to the study day.

At the study day, following an 8-h minimum overnight fast, a cannula was inserted into a suitable vein in one arm and an i.v. dose (∼1.3 mL, target 500 nmol) of the calcium-L-mefolinate-13C5 was prepared for administration into the noncannulated arm after warming a single-dose ampoule to room temperature. The dose was administered into a suitable vein via butterfly needle over a period of 5 s. A timer was started following a small saline flush (∼1 mL) through the line and needle.

Blood samples (5-mL volume) were obtained by cannula at the following time points: 2, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100, and 120 min. Blood was collected into 5-mL lithium heparin vacutainer tubes and mixed by inversion. Tubes were held at 4°C before centrifugation in batches at 2000 × g for 10 min at 4°C. Aliquots of plasma (500 µL) were transferred into 3 × 2-mL amber Eppendorf tubes, snap-frozen over dry ice, and stored at −80°C prior to LC-MS/MS analysis.

LC-MS/MS folate analysis methodology. A new method for the quantitative analysis of plasma folates was developed specifically for this study to allow rapid analysis of all the relevant folate forms, including 13C isotopes. The method used isotopic mobile phase conditions that resulted in an overall run time of <7 min for the analysis of folic acid, 5-methyltetrahydrofolic acid, 5-formyltetrahydrofolic acid, and their 13C5 isotopically labeled versions. Both electrospray positive and negative modes of MS were evaluated for detection and it was found that for the key folates, the positive mode gave the best MS sensitivity and the lowest background interference.

Preparation of calibrators for LC-MS/MS. Folates used for calibration were purchased from Schircks Laboratories. Labeled folates, (6S)-[13C5]
5-methyltetrahydrofolic acid-calcium salt (calcium-L-melolinate $^{13}$C$_5$), (6S)-$^{13}$C$_5$-5-formyltetrahydrofolic acid-calcium salt (calcium-L-leucovorin $^{13}$C$_5$), and $^{13}$C$_5$ folic acid were purchased from Merck Eprova. Briefly, powdered folates (0.2 g/L) were dissolved in 20 mmol/L potassium phosphate buffer, pH 7.2, containing 0.1% L-cysteine to prepare stock I, except for folic acid, which was prepared in 20 mmol/L potassium phosphate buffer without L-cysteine.

A portion of the solution was removed for spectrophotometric analysis and to the remaining volume of stock I, 1% (wt:v) ascorbic acid was added.

**Sample extraction.** The sample preparation method was based on that described by Garratt et al. (15). Briefly, to 500 μL of frozen plasma sample, 12 μL internal standard (5 μmol/L methotrexate) was added, followed by 1.488 mL cold buffered organic extraction solvent (5% concentrated stock buffer; 95% methanol). The concentrated stock buffer was comprised of 0.075 mol/L potassium dihydrogen phosphate, 0.4 mol/L ascorbic acid, and 0.8% (v/v) 2-mercaptoethanol, pH 6.0. Tube contents were vortex-mixed for 1 min to obtain a homogeneous solution and then centrifuged at 16,000 × g for 10 min. The supernatant was transferred to a clean tube and evaporated to dryness in a vacuum centrifuge. Dried extracts were reconstituted in 0.5 mL aqueous reconstitution buffer (comprised of 0.075 mol/L potassium dihydrogen phosphate, 0.052 mol/L ascorbic acid, and 0.1% (v/v) 2-mercaptoethanol, pH 6.0), vortex-mixed, then filtered through a 0.45-μm Whatman VectaSpin polypropylene microfilter by centrifugation at 16,000 × g for 10 min. The supernatant was transferred to an amber vial for analysis.

**LC-MS/MS conditions.** The extract was injected (10 μL) for LC-MS/MS analysis at a flow rate of 0.3 mL/min using a Shimadzu 10AD VP system equipped with binary pumps, a vacuum degasser, and a SIL-HTc autosampler (maintained at 4°C) using a Phenomenex Luna C18(2) 110 Å analytical column (100 × 2 mm; 3-μm particle size) maintained at 40°C. The HPLC mobile phase was isocratic and comprised 0.5% (v/v) acetic acid, 5% (v/v) acetonitrile, 10% (v/v) methanol, and 84.5% (v/v) water at pH 2.9.

All MS experiments were conducted on a 4000 QTRAP hybrid triple-quadrupole linear ion trap mass spectrometer (Applied Biosystems) equipped with a Turbolon source used in positive ion electrospray mode. A Windows XP (Microsoft) workstation running Analyst (version 1.4.1) was used for data acquisition and processing.

MRM parameters (precursor and product ion pairs, declustering potential, collision cell exit potential, and collision energy) were optimized by software automation while infusing a mixture of all folate standards (500 μg/L) at 200 μL/min with the MS peak widths set to 1.0 Th. Source parameters were optimized during infusion experiments at the working flow rate and were as follows: curtain, Gas 1, and 2 were 20, 40, and 20, respectively (arbitrary units). The ion source potential was 5.2 kV and the source was held at 350°C. Quantification was performed using Analyst 1.4.1, in Quantitative mode. LC-MS/MS settings for folate peak detection are summarized in Table 1.

**Determination of apparent volume of distribution for L-5-methyl-THF.** The data generated from the study were fit to two competing models that contained two and three compartments, respectively, using the SAAMII software package (17). After carrying out extensive goodness of fit tests (e.g., comparing the uncertainty of the model parameters, the randomness of the residuals, and the magnitude of the Akaike Information Criterion), it was decided that the two compartment model best represented the data. The notation used for the rate constants (Fig. 1) is that $k_i$ ($i$ represents the transfer of material (per unit time) from compartment $i$ to compartment “$i$-1.” The optimization routine of the software was used to estimate the $V_c$ and rate constants in the 2-compartment system (Fig. 1). The apparent volume of distribution was estimated using a model independent approach (10). It can be shown that:

**TABLE 1** Conditions and settings for folate peak detection by LC-MS/MS in plasma

<table>
<thead>
<tr>
<th>Folate</th>
<th>Retention time, min</th>
<th>Precursor ion, m/z</th>
<th>Product ion, m/z</th>
<th>DP, V</th>
<th>CE, V</th>
<th>CXP, V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic acid</td>
<td>2.22</td>
<td>442</td>
<td>295</td>
<td>76</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>$^{13}$C$_5$ folic acid</td>
<td>2.21</td>
<td>447</td>
<td>295</td>
<td>66</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>5-Methyl-THF</td>
<td>2.74</td>
<td>460</td>
<td>313</td>
<td>40</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td>$^{13}$C$_5$-5-methyl-THF</td>
<td>2.73</td>
<td>465</td>
<td>313</td>
<td>56</td>
<td>29</td>
<td>20</td>
</tr>
<tr>
<td>5-Formyl-THF</td>
<td>2.1</td>
<td>474</td>
<td>327</td>
<td>41</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td>$^{13}$C$_5$-5-formyl-THF</td>
<td>2.1</td>
<td>479</td>
<td>327</td>
<td>60</td>
<td>29</td>
<td>22</td>
</tr>
<tr>
<td>Methotrexate (internal standard)</td>
<td>4.38</td>
<td>455</td>
<td>308</td>
<td>91</td>
<td>29</td>
<td>8</td>
</tr>
</tbody>
</table>

1 CE, collision energy; CXP, collision cell exit potential; DP, declustering potential; LC-MS/MS, liquid chromatography-tandem MS; 5-methyl-THF, 5-methyltetrahydrofolic acid; 5-formyl-THF, 5-formyltetrahydrofolic acid; V, volts.
state. 5-methyl-THF, 5-methyltetrahydrofolic acid.

\[ V_{ss} = \frac{D}{C(0)} \]

where \( D \) is the mass (in nmol) of L-5-methyl-THF in the i.v. dose and \( C \) is the concentration (in nmol/L) of labeled L-5-methyl-THF in the plasma at time, \( t \). The AUC and AUMC were both estimated by the SAAMII software in numerical mode.

It can also be shown (10) that \( V_{ss} \) is equal to the sum of the volumes of the compartments in a multi-compartment system (i.e., \( V_{ss} = V_1 + V_2 \)). The volume of the second compartment \( V_2 \) is equal to:

\[ V_2 = \frac{k(2,1)}{k(1,2)} \]

and therefore,

\[ V_{ss} = V_1 \left( \frac{k(1,2) + k(2,1)}{k(1,2)} \right) \]

**Pool size estimation.** The validity of this approach depends on the assumption that the system is linear and that elimination takes place primarily from the plasma. The mass of folate contained in compartments 1 and 2 is calculated as follows. If the volume of distribution in compartment 1 is \( V_1 \) and the mean plasma concentration of unlabeled L-5-methyl-THF is \( \text{Conc}_{\text{SMTHF}} \), then the mass of folate in compartment 1 (\( M_1 \)) is given by:

\[ M_1 = \text{Conc}_{\text{SMTHF}} \cdot V_1 \]

Similarly, the mass of folate in compartment 2 (\( M_2 \)) is given by:

\[ M_2 = M_1 \cdot \frac{k(2,1)}{k(1,2)} \]

**Assessment of RBC folate status.** Whole blood was hemolyzed by 10-fold dilution in 1% ascorbic acid preadjusted to pH 4.25 (18). Samples were then analyzed using a glyceral-mediated, cryo-preserved (19,20), and chloramphenicol-resistant *Lactobacillus rhamnosus* NCIMB 10463 microbiological assay (18). RBC folate was calculated from whole blood values using individual hematocrits and after correction for plasma folate concentration.

**Assessment of MTHFR genotype.** To identify the MTHFR C677T (rs1801133) genotype, DNA was extracted from whole blood collected in EDTA tubes using standard methods and sub aliquotted onto 96-well plates. PCR assays to amplify MTHFR were performed on 100 ng DNA by using 200 nmol/L of each primer (sense GGTCAAGAAGCATAT- CAGTCATG; antisense CACAAAGCGGAAGAATGTGTC) and 1× PCR Thermo Start Mastermix (ABgene UK) in a reaction volume of 25 µL. PCR reactions were carried out with an annealing temperature of 55°C with 38 cycles of amplification. Restriction digests were then carried out on the PCR products by using 0.2 units in 20 µL reaction of *HinII*. Digests were electrophoresed on a 1× Tris/borate/EDTA 3% Metaphor agarose gel (FMC Bioproducts) at 80 V for 50 min and gene variation was assessed by fragment size.

**Statistical analysis.** Data are reported as means ± SD. Statistical analyses were performed using the R data analysis software (21). Parametric models (linear regression and ANOVA) were used to analyze the data. Diagnostic checks were performed to ensure that the use of linear models was valid. This required the models to satisfy the usual assumptions that regression analysis is based on and included checking the normality of residuals and ensuring that the variance of the residuals was similar for all values of the explanatory variable. The removal of outliers was necessary for points with high leverage. No transformation of either the response or explanatory variables was required. All results were considered significant if \( P < 0.05 \) (2-sided tests). In all analyses, \( V_{ss} \) was set as the response variable and genotype, age, weight, BMI, blood pressure, hematocrit, RBC folate concentration, and unlabeled 5-methyltetrahydrofolic acid plasma concentration were set as single explanatory variables.

**Results**

A total of 26 volunteers signed consent and were screened for the study and 7 were excluded at this screening stage. One volunteer was excluded on the study day due to the difficulty in cannulating their vein. Thus, a total of 18 volunteers participated in the study day. Data from 2 volunteers were excluded from final analysis due to cannulation problems, resulting in lack of complete timepoint samples being collected. One additional volunteer was excluded from analysis, leaving data for 15 volunteers (11 males, 4 females; mean age 47 ± 12 y) (Table 2; Supplemental Table 1). The plasma concentration of [13C6]5-methyltetrahydrofolic acid was measured over time by LC-MS/MS (Fig. 2). There was rapid clearance from the plasma during the first 20 min and then a slower decay of enrichment over the next 100 min. Visually, the model fit to the experimental data was good (Fig. 2). The uncertainty on the model parameters was generally <10% (RSD; data not shown). The uncertainty in the mean concentration measured of the labeled L-5-methyl-THF was higher during the first 20 min, which is to be expected in a highly dynamic system.

The LC-MS/MS assay method was linear over the range 0.5 to 320 nmol/L in human plasma and showed recovery values (of folates added to plasma) between 68 and 81% (at 30 nmol/L).

**TABLE 2** Characteristics of study participants

<table>
<thead>
<tr>
<th>Age, y</th>
<th>47 ± 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, n</td>
<td>4</td>
</tr>
<tr>
<td>Male, n</td>
<td>11</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.73 ± 0.09</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>80.6 ± 12.0</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.2 ± 2.2</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>134 ± 12</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>82 ± 6</td>
</tr>
<tr>
<td>Hematocrit, v/v</td>
<td>0.43 ± 0.04</td>
</tr>
<tr>
<td>RBC folate, µmol/L</td>
<td>1.1 ± 0.4</td>
</tr>
</tbody>
</table>

1 Values are mean ± SD, n = 15. Individual data are shown in Supplemental Table 1. Data were obtained for age, height, weight, BMI, and blood pressure on the study day. Samples for measuring hematocrit and RBC folate were obtained at screening.
and 70 and 82% (at 300 nmol/L) for all folates analyzed. Within-day precision was 2.3–8.4% (at 30 nmol/L) and 1.4–5.4% (at 300 nmol/L) and within-day accuracy was within 15% for all folate analytes. Ion suppression values (matrix effects) were measured at the lower validation concentration of 30 nmol/L in plasma and showed slight, but acceptable, suppression of signals (71–89% compared with 100% standards of each folate). The LLOQ of folates in plasma was 0.25–0.5 nmol/L, an improved value compared with the limit of detection reported in previous methods (15,22). The internal standard (methotrexate) spiked at its standard concentration in human plasma ($n = 5$), gave a recovery of 84.5%, a precision of RSD 6.6%, and ion suppression of 84.8%.

Data for the mass of infused i.v. dose, modeled rate constants, estimated pool sizes, apparent volume of distribution, and mean unlabeled plasma 5-methyltetrahydrofolate acid concentration are given in Table 3. The mean $V_{ss}$ of 392 ± 110 mL/kg (31.4 L for ~80 kg body weight) in the current study was similar to the 389 mL/kg used in our previous study (8). The apparent volume of distribution was correlated with body weight ($r = 0.64$; $P = 0.01$). The regression equation relating $V_w$ (L) to body weight (kg) was:

$$V_w = 0.623 \times \text{bodyweight} - 18.18.$$ 

The relationship between apparent volume of distribution and body weight is shown in Figure 3. The 95% prediction interval is also shown.

Apparent volume of distribution was not significantly correlated with MTHFR C677T genotype, age, height, BMI, diastolic blood pressure, systolic blood pressure, hematocrit, RBC folate concentration, or unlabeled plasma folate concentration.

**Discussion**

In the present study, the apparent volume of distribution at steady state was estimated to be 32.0 L (392 ± 110 mL/kg body weight) using a physiological i.v. dose (440 nmol) of a $^{13}$C stable isotope-labeled version of the naturally circulating plasma folate, 5-methyltetrahydrofolate. The rationale for this work was 2-fold. There was a lack of consensus in the literature as to what the true value of the apparent volume of distribution was and there was no simple equation that could be used to predict the volume of distribution from an easily measurable (noninvasive) parameter (10–12,23).

There are no previous reports describing the estimation of the apparent volume of distribution from 5-methyltetrahydrofolate i.v. doses in healthy adults, although clearance of radiolabeled 5-methyltetrahydrofolate in pernicious anemia patients has been described (24). However, there are a number of reports of estimates of apparent volume of distribution using doses of 5-formyltetrahydrofolate (11,12,23). This is due to the use of this folate species (pharmaceutically known as leucovorin or folinic acid) as a folate-rescue therapy during antifolate chemotherapy regimens. In these papers, the original folate form (5-formyltetrahydrofolate) in the dose appeared in the plasma mainly as 5-methyltetrahydrofolate (some appears as unconverted 5-formyltetrahydrofolate) and the apparent volume of distribution was estimated to be $V_{ss} = 15.5$ L.
after a 25-mg dose (11), \( V_\beta = 22.9 \) L after doses ranging from 25 to 100 mg (12), and \( V_\beta = 37.6 \) L after a 20-mg dose (23). A study by Loew et al. (13) gave a 1.1-mg i.m. dose of folic acid and estimated the apparent volume of distribution (\( V_\alpha \)) as \(-31\) L for an 80-kg person (389 mL/kg body weight). There is clearly no consensus in the literature as to the true value of the apparent volume of distribution for L-5-methyl-THF.

The present study was designed to overcome the inherent problems in the previous work and has several advantages: 1) the injected folate dose contained the major circulating folate form in the body (L-5-methyl-THF); 2) the injected folate was labeled and easily distinguishable in the plasma from the unlabeled (endogenous) L-5-methyl-THF by LC-MS/MS; and 3) the injected folate dose was small [440 nmol (0.2 mg)] and therefore likely to follow normal kinetics in the body.

All three of these advantages should have led to a more accurate estimation of the apparent volume of distribution compared to those previously reported in the literature. The volume from the present work is derived from the estimation of the volume at steady state (\( V_\alpha \)) rather than from the estimation of the volume using the rate constant for elimination from the body (\( V_\beta \)). Other work has indicated that \( V_\beta \) is time varying and is therefore dependent on the length of the experiment used to measure it (10). For this reason, \( V_\alpha \) is presented in Table 3. \( V_\beta \) was also estimated (35.9 ± 13.9 L).

Another result from the present study is the development of a simple equation relating \( V_\alpha \) to body weight (see Results; Fig. 3). The predictive ability of the regression equation has not been rigorously tested due to the lack of independent data that would be required for validation. Given the relatively small amount of data used to build the model and the wide 95% prediction intervals, some care will be needed when using the equation to predict apparent volume of distribution from body weight in future studies. Models for prediction of apparent absorption of folate test doses rely on an estimation of \( V_\alpha \) and these models will be more accessible to researchers if only simple anthropometric measurements are required to estimate key parameters.

In addition to the calculation of the apparent volume of distribution, several other kinetic parameters were estimated in the present study. The mass of folate in the sampled compartment (pool 1) was 174 ± 91 nmol and that in the other compartment (pool 2) was 449 ± 349 nmol. These are highly dynamic pools and probably represent the exchange of free folate in the plasma with folate attached to binding proteins in the plasma, respectively. The total body pool of folate is estimated to be in the range of 50,000-225,000 nmol (25,26), so clearly the pools in the present study are just a small fraction of the total folate contained in the body.

Unlabeled (endogenous) 5-methyltetrahydrofolate was also measured and its mean value over the study period is presented in Table 3. There was no apparent perturbation (defined as an increase of >10% above the baseline value) of the endogenous plasma folate pool in contrast to when folate doses were administered orally (27). The lack of perturbation is probably due to the smaller amount of folate that the liver would initially encounter after an i.v. dose compared to that absorbed from the oral dose, because all the folate from an absorbed oral dose would have to pass through the liver on first pass from the portal vein.

Previous modeling work on estimating short-term kinetics and bioavailability of folate has required knowledge of the apparent volume of distribution (8,28,29). These papers used the estimate of apparent volume of distribution from the work of Loew et al. (13), which was calculated to be 389 mL/kg body weight. Although this was derived after a large unlabeled dose (2300 nmol) of folic acid, it has been shown to be very similar to the estimate from the present work (392 mL/kg body weight). This leads to the conclusion that previous estimates of apparent absorption of folate do not require any adjustment in light of findings from the present work.

Future studies need to focus on estimates of the first pass effect for folic acid and L-5-methyl-THF oral test doses. If a simple equation can be developed that relates an easily measurable parameter (e.g., RBC folate concentration) to the fraction of folate that is removed by the liver on first pass, then the true absorption of a test dose of folate can be estimated from the appearance of labeled L-5-methyl-THF in the plasma.

In conclusion, the current study estimates the apparent volume of distribution for 5-methyltetrahydrofolate in healthy participants. The variation in \( V_\alpha \) between individuals was significantly associated with body weight but could not be explained by correlations with MTHFR C677T genotype, age, height, BMI, blood pressure, hematocrit, RBC folate concentration, or unlabeled plasma folate concentration. Furthermore, this apparent volume of distribution is similar to that used in a previously published mathematical model and therefore strengthens the results found in that work. The linear relationship between body weight and apparent volume of distribution will make it easier to estimate apparent folate absorption in future studies.

Acknowledgments

M.J.K., J.R.D., A.J.A.W., and P.M.F. designed the research; M.J.K., E.I.B., and D.A.B. conducted research; E.I.B., D.A.B., and J.R.D. analyzed data; M.J.K., D.A.B., J.R.D., and A.J.A.W. wrote the paper; and P.M.F. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited