Human Nutrition and Metabolism

Conversion of 5-Formyltetrahydrofolic Acid to 5-Methyltetrahydrofolic Acid Is Unimpaired in Folate-Adequate Persons Homozygous for the C677T Mutation in the Methylenetetrahydrofolate Reductase Gene

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ABSTRACT Methylenetetrahydrofolate reductase (MTHFR) catalyzes the synthesis of 5-methyltetrahydrofolinic acid (5-CH₃-H₄ folic acid), the methyl donor for the formation of methionine from homocysteine. A common C677T transition in the MTHFR gene results in a variant with a lower specific activity and a greater sensitivity to heat than the normal enzyme, as measured in vitro. This study was undertaken to determine the capacity of homozygotes for the MTHFR C677T transition to convert 5-formyltetrahydrofolic acid (5-HCO-H₄ folic acid) to 5-CH₃-H₄ folic acid, a process that requires the action of MTHFR. Six subjects homozygous for the C677T transition (T/T) and 6 subjects with wild-type MTHFR (C/C) were given a 5-mg oral dose of (6R,S)-5-HCO-H₄ folic acid. Plasma and urine were analyzed for 5-CH₃-H₄ folic acid concentrations using affinity/HPLC coupled with fluorescence or UV detection. The mean areas under the curves created by the rise and fall of plasma 5-CH₃-H₄ folic acid after the oral dose did not differ between the two genotypes, 424.5 ± 140.3 T/T vs. 424.1 ± 202.4 nmol/L C/C. There was also no significant difference in the mean cumulative 7-h urinary excretion of 5-CH₃-H₄ folic acid between the T/T (2.5 ± 1.4 μmol) and C/C (1.9 ± 1.0 μmol) genotypes. Under the conditions employed, the conversion of oral 5-HCO-H₄ folic acid to 5-CH₃-H₄ folic acid is not impaired in persons with the T/T MTHFR genotype. Possible reasons for these findings are discussed.


KEY WORDS: folate • methylenetetrahydrofolic acid • formyltetrahydrofolic acid • homocysteine • polymorphism • humans

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolic acid (5-CH₃-H₄ folic acid), which is the predominant form of folate in blood and the methyl donor for the conversion of homocysteine to methionine. Rare inherited defects in MTHFR result in hyperhomocysteinemia and low-to-normal plasma concentrations of methionine. The clinical manifestations of this deficiency vary in a range of neurologic and vascular abnormalities, and the severity of these abnormalities correlates with the degree of loss in enzyme activity (Kanwar et al. 1976, Mudd et al. 1972, Rosenberg 1989, Wong et al. 1977).

Kang et al. (1988) first identified a common variant of the MTHFR enzyme, characterized by reduced enzyme activity (~50%) and greater sensitivity to heat inactivation compared with the normal enzyme in vitro. When lymphocyte or fibroblast extracts containing this variant enzyme were preincubated at 46°C for 5 min, the residual enzyme activity was considerably lower than the normal enzyme; thus this variant was named “Thermolabile MTHFR.”

Upon isolation of the human cDNA for MTHFR (Goyette et al. 1994), these in vitro properties of the thermolabile enzyme were found to be due to a homozygous cytosine-to-thymidine transition at nucleotide 677 (C677T), resulting in an alanine-to-valine substitution (Frost et al. 1995). The prevalence of this mutation was first described in French Canadians with an allele frequency of 38% and homozygosity (i.e., T/T genotype) of 12%. More recently, the frequency of the T allele has been determined in Caucasians (36%), Asians (40%), and African Blacks (5%) (Franco et al. 1998).

To date, the activity of thermolabile MTHFR has been described only in vitro. The intent of this study, therefore, was to determine whether the presence of this polymorphism impairs the synthesis of 5-CH₃-H₄ folic acid in vivo. We determined the rise in 5-CH₃-H₄ folic acid concentration in plasma and urine after oral ingestion of 5-HCO-H₄ folic acid in human subjects with either the T/T (mutant) or C/C (wild-type) MTHFR genotype. Our approach was based on previous studies (Nixon and Bertino 1972, Whitehead et al. 1972),
which have shown that such an increase in plasma and urinary 5-CH$_2$CH$_3$H$_4$ folate follows the conversion of 5-HCO-H$_4$ folic acid to 5-CH$_2$CH$_3$H$_4$ folate, a process that requires the action of MTHFR.

**SUBJECTS AND METHODS**

The study protocol was approved by the Human Investigation Review Committee of Tufts-New England Medical Center. Four female and 2 male subjects homozygous for the MTHFR C677T mutation and between 25 and 75 y old were matched by gender and age (within 1 y) with wild-type genotype individuals. All subjects were healthy and well nourished as assessed by an overall physical examination performed before enrollment. Subjects were instructed to avoid any vitamin supplements for 2 wk before study participation. Upon admission to the Metabolic Research Unit, only low folate meals, snacks and beverages were provided. After an overnight fast, baseline blood and urine samples were collected. A 5-mg oral dose of (6R,S)-5-HCO-H$_4$ folic acid was then administered with water, and urine was collected at 30 min and 1 h postdose, then every hour for a total of 7 h. Urine collection containers contained 1 gm sodium ascorbate as a preservative and were kept at 4°C during the course of the collection. Samples were immediately processed and stored at ~70°C until analysis. Fasting total plasma and RBC folates were determined by a conventional microbial (Lactobacillus casei) method using a 96-well plate (Tamura et al. 1990). Fasting plasma total homocysteine was determined by HPLC using the method of Araki and Sako (1987). Specific forms of plasma and urine folates were analyzed by a combined affinity/HPLC method (Bagley and Selhub 1997) and are described in more detail below. All chemicals were purchased from Sigma Chemical (St. Louis, MO) unless otherwise indicated.

**MTHFR genotype determination.** Analysis of the MTHFR C677T mutation was based on the method of Frooss et al. (1995) with minor modifications. Briefly, DNA was isolated from peripheral leukocytes using an Invitrogen Easy-DNA Kit (San Diego, CA). Polymerase chain reaction was performed using genomic DNA and the primers 5'-TGAAGGAGAA GGTGTCTGCG GGA-3' and 5'-AGGACGGTGCC GGTGAGAGTG-3' to generate a 198-bp fragment. The amplified product was digested with HinfI restriction enzyme (Life Technologies, Grand Island, NY), which cleaves only the mutant MTHFR allele into 175- and 23-bp fragments. Restriction analysis was visualized by agarose gel electrophoresis with ethidium bromide staining.

**Analysis of plasma folates.** For the extraction of plasma folate, an aliquot of plasma (0.2 mL) was added to 0.8 mL of a high pH (9.4) buffer containing 0.1 mol/L potassium tetraborate, 2 g/L triton X-100, 1 g/L sodium ascorbate and 10.9 mmol/L of an internal standard, 5-ethyltetrahydrofolic acid (5-CH$_2$CH$_3$H$_4$ folic acid), synthesized in our laboratory (see below). This solution was heated at 100°C for 25 min, then centrifuged at 16,000 × g. We used a Gilson Aspec XL solid phase extractor (Middleton, WI) to perform the following step sequence: 0.8 mL aliquot of supernatant was applied to a 1-mL capacity affinity column containing 0.2 mL Sepharose matrix bound to purified milk folate binding protein (Selhub et al. 1988), which affinity column was washed with a 1 mol/L potassium phosphate solution (pH 7) and water; folates were eluted with 0.7 mL of 20 mmol/L trifluoroacetic acid and 1 g/L ascorbic acid into a tube containing 1 mol/L piperazine (0.01 mL), which allowed neutralization of the eluant to pH 7; 0.5 mL of eluate was injected onto an ODS Hypersil 250 × 4.6 mm (Keystone, Bellefonte, PA) analytical column. We used an isocratic system (30 mmol/L potassium phosphate, 115 mL/L acetonitrile, pH 2.4) to separate methyl- from ethyltetrahydrofolic acid. Fluorescence (Applied Biosystems 980 detector, Foster City, CA) was used for detection because of its high sensitivity for 5-CH$_2$CH$_3$H$_4$ folic acid (Gregory et al. 1984). Millenium software (Millipore Corporation, Milford, MA) was used for peak integration.

**Synthesis of 5-ethyltetrahydrofolic acid standard.** The measurement of plasma folates was automated such that the affinity column eluate was injected immediately onto the analytical column by the Gilson instrument. Therefore, it was necessary to use an internal standard that would allow quantification of folate recovery from the affinity column during the HPLC phase of folate determination. For this purpose, we synthesized 5-CH$_2$CH$_3$H$_4$ folic acid, which was detectable by fluorescence and did not coelute with 5-CH$_2$CH$_3$H$_4$ folate, the predominant folate form in plasma. One milligram of tetrathydrofolic acid (1 mL of a 2 mmol/L solution) was added to a tube containing acetaldehyde (0.05 mL) and 2-mercaptoethanol (0.02 mL). This solution was cooled on ice; solid potassium borohydride (25 mg) was added, and the tube was capped with a rubber stopper in which a syringe needle was inserted. The tube was incubated at 37°C for 1 h. The solution was cooled, then neutralized with glacial acetic acid. The newly synthesized 5-CH$_2$CH$_3$H$_4$ folic acid was purified by the ion-pair HPLC method described by Selhub (1989). Treatment with TFA pH 4.2 for 15 min followed by HPLC showed no contaminating peak by this method.

**Analysis of urine folates.** Folates were analyzed in urine samples collected at baseline and in the urine pooled for each subject from the cumulative 7-h collection after 5-mg-H$_2$ folate ingestion. The same affinity/HPLC principle was used as for plasma folate determination; however, urine folate analysis was performed using a nonautomated HPLC system to accommodate a larger sample volume. This larger volume enabled the detection of post-dose accumulation of 5-HCO-H$_4$ folic acid by UV, which has weak fluorescence. Compared with fluorescence, however, the limits of detection for 5-CH$_2$CH$_3$H$_4$ folic acid by UV light are ~1000-fold lower (Bagley and Selhub 1997, Gregory et al. 1984), resulting in the inability to detect folates in the urine samples collected at baseline. An aliquot of urine (5 mL) was heat-extracted with 5 mL of the same high pH buffer used for plasma folate analysis. After centrifugation, the supernatant was mixed with [1H]-folate acid tracer for quantification of folate recovery (Varela-Moreiras et al. 1992), then applied onto a 1-mL (bed volume) sepharose-folate binding protein column. After being washed with 1 mol/L potassium phosphate (pH 7) and water, the column was eluted with 3 mL of an acidic solution (20 mmol/L trifluoroacetic acid, 1 g/L ascorbic acid). The acid eluate was neutralized and a portion (2 mL) was applied onto a Bio-sil ODS-55 150 × 4 mm (Bio-Rad, Richmond, CA) analytical column connected to a Hewlett Packard 1090 HPLC system (Palo Alto, CA). Folate forms were separated using a tetrabutyl ammonium phosphate (43 mmol/L)/sodium chloride (22.7 mmol/L) solution buffered to pH 6.8 with an acetomitrile gradient. In this HPLC system, individual folate forms are identified on the basis of retention time and their specific spectra. Folate quantification is based on peak areas and peak activity coefficient determined for each folate form (Selhub 1989). Sample peaks were integrated by Hewlett Packard Chemstation software v.A.02.02 (Avondale, PA).

**Statistical analysis.** The area under the curve (AUC) created by the rise and fall in plasma 5-CH$_2$CH$_3$H$_4$ folic acid over 7 h after the 5-HCO-H$_4$ folic acid dose was calculated for each subject by the trapezoidal rule. The paired Student’s t test was then used to compare the mean differences in folate status indices, the AUC for each genotype and total urinary 5-CH$_2$CH$_3$H$_4$ folate acid excreted during the 7-h testing period. ANOVA was used to test for interactions of age or sex with either genotype or the plasma 5-CH$_2$CH$_3$H$_4$ folate acid AUC after the 5-HCO-H$_4$ folate acid dose. All statistical analyses were performed with Systat 5.2.1 Software for Macintosh (Evanton, IL). Differences were significant at $P < 0.05$. Values are expressed as means ± SD.

**RESULTS**

The age and gender distribution between the T/T and C/C MTHFR genotype subjects was the same due to pairing of subjects. Notably, indices of folate status (i.e., fasting total plasma folate, RBC folate and homocysteine concentrations) were not different between the two groups and are considered normal (Table 1).

As demonstrated in representative chromatograms from one study subject, 5-CH$_2$CH$_3$H$_4$ folic acid was the predominant folate form at baseline (Fig. 1); 1 h after the 5-mg oral dose of 5-HCO-H$_4$ folic acid, the 5-CH$_2$CH$_3$H$_4$ folic acid peak height rose fivefold over baseline. The 5-CH$_2$CH$_3$H$_4$ folic acid peak
eluted at \(5.6\) min and was equivalent to \(20\) nmol/L at baseline and \(100\) nmol/L at \(1\) h.

Plasma 5-CH\(_3\)-H\(_4\) folic acid concentrations in all subjects peaked within \(3\) h and returned to close to baseline after the 7-h testing period (Fig. 2). The mean increase in the AUC for plasma 5-CH\(_3\)-H\(_4\) folic acid was \(424.5 \pm 140.3\) h nmol/L in the T/T genotype subjects and \(424.1 \pm 202.4\) h nmol/L in the C/C genotype group.

As shown in representative chromatograms from one study subject, neither 5-CH\(_3\)-H\(_4\) folic acid nor 5-HCO-H\(_4\) folic acid was detectable in the urine at baseline (Fig. 3). However, both folate forms were detectable in the total volume of urine collected during the 7 h after the oral 5-HCO-H\(_4\) folic acid dose. In this subject, the cumulative 5-HCO-H\(_4\) folic acid peak area at a retention time of \(18.8\) min was equivalent to \(1.4\) \(\mu\)mol, and the 5-CH\(_3\)-H\(_4\) folic acid peak area at a retention time of \(19.5\) min was equivalent to \(2.3\) \(\mu\)mol.

The mean 5-CH\(_3\)-H\(_4\) folic acid excreted by the T/T genotype subjects was \(2.5 \pm 1.4\) \(\mu\)mol compared with \(2.0 \pm 1.0\) \(\mu\)mol in subjects with the wild-type enzyme, \(P > 0.4\) (Table 2). These quantities represent \(\sim 45\%\) of the biologically active \((6\text{S})\)-5-HCO-H\(_4\) folic acid administered. Total urinary excretion of 5-HCO-H\(_4\) folic acid accounted for an additional 10\% of the total dose. Mean urinary excretion of 5-HCO-H\(_4\) folic acid in the T/T genotype subjects, \(1.0 \pm 0.7\) \(\mu\)mol, was not different from that in the C/C genotype subjects, \(1.0 \pm 0.6\) \(\mu\)mol.

There was no interaction of age or sex with either genotype or the response in plasma 5-CH\(_3\)-H\(_4\) folic acid levels after the 5-HCO-H\(_4\) folic acid dose (data not shown). Urinary excretion of 5-CH\(_3\)-H\(_4\) folic acid was lower in elderly subjects compared with the young, reflecting the general decline in renal clearance with age. However, this difference did not affect the final analysis due to pairing of subjects.

### TABLE 1
Characteristics of study participants according to methylenetetrahydrofolate reductase (MTHFR) genotype: homozygous for the C677T MTHFR polymorphism (T/T) and wild-type MTHFR (C/C)\(^1\)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>T/T</th>
<th>C/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>54.9 ± 20.8</td>
<td>54.7 ± 20.8</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>2/4</td>
<td>2/4</td>
</tr>
<tr>
<td>Plasma folate, nmol/L</td>
<td>23.3 ± 12.4</td>
<td>23.2 ± 14.1</td>
</tr>
<tr>
<td>RBC folate, pmol/mg hemoglobin</td>
<td>2.6 ± 1.3</td>
<td>2.7 ± 1.3</td>
</tr>
<tr>
<td>Plasma homocysteine, (\mu)mol/L</td>
<td>5.8 ± 2.6</td>
<td>5.4 ± 2.5</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± sd, \(n = 6\), and are not different between genotypes (paired Student's \(t\) test \(P > 0.05\)).
At baseline, 5-HCO-H₄ folic acid and 5-CH₃-H₄ folic acid were not acid. Evidence that the intestinal tissue has the capacity of converting folic acid to a biologically active form before the ingested folate reached the liver, i.e., before the ingestion of the radiolabeled 5-[14C]-HCO-[3H]-THF, was sufficient under the described conditions to prevent us from observing any decrease in the appearance of 5-CH₃-H₄ folic acid. Several explanations can be used to interpret these results.

One possibility is that the T/T MTHFR variant was protected from inactivation in the presence of adequate folate substrate concentrations. Such an interpretation has been derived from recent epidemiologic studies (Christensen et al. 1997, Jacques et al. 1996, Schwartz et al. 1997, Verhoef et al. 1997), which show that plasma total homocysteine in persons with the T/T MTHFR genotype is elevated only in those with a low folate status. When folate status is adequate, total homocysteine levels are not different from those in persons with the wild-type genotype. Because homocysteine is a substrate for methylation by 5-CH₃-H₄ folic acid, the level of plasma homocysteine in individuals with the T/T MTHFR genotype: homozygous for the C677T MTHFR polymorphism according to methylenetetrahydrofolate reductase (MTHFR) genotype; homocysteine for the C677T MTHFR polymorphism (T/T) and wild-type MTHFR (C/C); 1,2

DISCUSSION

In this study we measured the capacity of individuals homozygous for the C677T MTHFR mutation (T/T) and wild-type (C/C) genotypes to convert 5-HCO-H₄ folic acid to 5-CH₃-H₄ folic acid, a process that requires the action of MTHFR. This conversion was assessed by the rise in 5-CH₃-H₄ folic acid concentrations in both plasma and urine after a 5-mg oral dose of 5-HCO-H₄ folic acid. An earlier study by Whitehead et al. (1972) showed that ingestion of 5-HCO-H₄ folic acid (2 mg) is associated with a prompt rise of 5-CH₃-H₄ folic acid in the portal and peripheral blood. The fact that this rise in 5-CH₃-H₄ folic acid was seen in the portal blood, i.e., before the ingested folate reached the liver, is evidence that the intestinal tissue has the capacity of converting 5-HCO-H₄ folic acid to 5-CH₃-H₄ folic acid. This capacity of the intestine to metabolize folates to the 5-methyl-derivative has also been demonstrated in ex vivo studies using rat intestinal tissue (Selhub et al. 1973, Strum 1977).

A second study by Nixon and Bertino (1972) used 5-[14C]-HCO-[¹H]-H₄ folic acid (0.18–2.65 μmol) to show that ingestion of this folate is associated with the prompt appearance of radioactivity in peripheral plasma that is proportional to the amount ingested and consists primarily (90%) of 5-CH₃-[¹H]-H₄ folic acid. These data indicate that in the course of absorption, there is a loss of the formyl group and subsequent acquisition of a new one-carbon unit, which is then reduced to the 5-methyl-derivative. This study also showed that after ingestion of the radiolabeled 5-[¹⁴C]-HCO-[¹H]-THF, the pattern of urinary folate excretion is characterized by an initial rise in 5-[¹⁴C]-HCO-[¹H]-THF followed by a rise in 5-CH₃-[¹H]-H₄ folic acid that is proportional to the levels in the plasma (Nixon and Bertino 1972). These data indicate that the source of these urinary folates is the ingested dose.

Two additional studies by McGuire et al. (1987) and Straw et al. (1984) investigated the pharmacokinetics of orally ingested (6R,S)-5-HCO-H₄ folic acid at high doses. Assuming that only the natural (6S)-isomer possesses biologic activity (Keresztesy and Silverman 1951), they showed that essentially 100% of the stereoreactive (6S)-isomer was absorbed, with >90% of the stereoreactive dose represented in the plasma as 5-CH₃-H₄ folic acid after oral ingestion of a 20- to 25-mg dose.

The present study is consistent with these previous studies in that the oral administration of 5-HCO-H₄ folic acid was associated with a prompt rise of 5-CH₃-H₄ folic acid concentrations in both plasma and urine. In plasma, this rise peaked between 1 and 3 h after which time the concentrations were approximately fivefold greater than baseline. In the urine, 5-CH₃-H₄ folic acid and 5-HCO-H₄ folic acid were initially undetectable but were markedly elevated after the oral dose. For the 5-CH₃-H₄ folic acid, the amount excreted was ~45% of the stereoreactive ingested dose. The amount of 5-HCO-H₄ folic acid excreted represented an additional 10% of the unmetabolized total dose.

Our data showed no difference between the C/C and T/T MTHFR genotypes with respect to the rise of 5-CH₃-H₄ folic acid concentrations in plasma and urine after the oral dose of 5-HCO-H₄ folic acid. This lack of difference between genotypes suggests that the activity of the mutant MTHFR variant is sufficient under the described conditions to prevent us from observing any decrease in the appearance of 5-CH₃-H₄ folic acid. Several explanations can be used to interpret these results.

In this study we measured the capacity of individuals homozygous for the C677T MTHFR mutation (T/T) and wild-type (C/C) genotypes to convert 5-HCO-H₄ folic acid to 5-CH₃-H₄ folic acid, a process that requires the action of MTHFR. This conversion was assessed by the rise in 5-CH₃-H₄ folic acid concentrations in both plasma and urine after a 5-mg oral dose of 5-HCO-H₄ folic acid. An earlier study by Whitehead et al. (1972) showed that ingestion of 5-HCO-H₄ folic acid (2 mg) is associated with a prompt rise of 5-CH₃-H₄ folic acid in the portal and peripheral blood. The fact that this rise in 5-CH₃-H₄ folic acid was seen in the portal blood, i.e., before the ingested folate reached the liver, is evidence that the intestinal tissue has the capacity of converting 5-HCO-H₄ folic acid to 5-CH₃-H₄ folic acid. This capacity of the intestine to metabolize folates to the 5-methyl-derivative has also been demonstrated in ex vivo studies using rat intestinal tissue (Selhub et al. 1973, Strum 1977).

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The present study is consistent with these previous studies in that the oral administration of 5-HCO-H₄ folic acid was associated with a prompt rise of 5-CH₃-H₄ folic acid concentrations in both plasma and urine. In plasma, this rise peaked between 1 and 3 h after which time the concentrations were approximately fivefold greater than baseline. In the urine, 5-CH₃-H₄ folic acid and 5-HCO-H₄ folic acid were initially undetectable but were markedly elevated after the oral dose. For the 5-CH₃-H₄ folic acid, the amount excreted was ~45% of the stereoreactive ingested dose. The amount of 5-HCO-H₄ folic acid excreted represented an additional 10% of the unmetabolized total dose.

Our data showed no difference between the C/C and T/T MTHFR genotypes with respect to the rise of 5-CH₃-H₄ folic acid concentrations in plasma and urine after the oral dose of 5-HCO-H₄ folic acid. This lack of difference between genotypes suggests that the activity of the mutant MTHFR variant is sufficient under the described conditions to prevent us from observing any decrease in the appearance of 5-CH₃-H₄ folic acid. Several explanations can be used to interpret these results.

One possibility is that the T/T MTHFR variant was protected from inactivation in the presence of adequate folate substrate concentrations. Such an interpretation has been derived from recent epidemiologic studies (Christensen et al. 1997, Jacques et al. 1996, Schwartz et al. 1997, Verhoef et al. 1997), which show that plasma total homocysteine in persons with the T/T MTHFR genotype is elevated only in those with a low folate status. When folate status is adequate, total homocysteine levels are not different from those in persons with the wild-type genotype. Because homocysteine is a substrate for methylation by 5-CH₃-H₄ folic acid, the level of plasma homocysteine in individuals with the T/T MTHFR genotype: homozygous for the C677T MTHFR polymorphism according to methylenetetrahydrofolate reductase (MTHFR) genotype; homocysteine for the C677T MTHFR polymorphism (T/T) and wild-type MTHFR (C/C); 1,2

**TABLE 2**

<table>
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<tr>
<th>Genotype</th>
<th>T/T</th>
<th>C/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-CH₃-H₄ folic acid, μmol</td>
<td>2.5 ± 1.4</td>
<td>1.9 ± 1.0</td>
</tr>
<tr>
<td>% of active dose</td>
<td>48</td>
<td>37</td>
</tr>
<tr>
<td>5-HCO-H₄ folic acid, μmol</td>
<td>1.0 ± 0.7</td>
<td>1.0 ± 0.7</td>
</tr>
<tr>
<td>% of total dose</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 6, and are not different between genotypes (paired Student’s t test P > 0.05).
2 Abbreviations used: 5-CH₃-H₄ folic acid, 5-methyltetrahydrofolate acid; 5-HCO-H₄ folic acid, (6R,S)-5-formyltetrahydrofolate acid.
3 Assumes that only the 6S-isomer of (6R,S)-5-HCO-H₄ folic acid is biologically active.
genotype serves to indicate whether the T/T MTHFR variant is capable of meeting the cellular demands for 5-CH$_3$H$_4$folic acid. Thus, when plasma homocysteine in homozygotes for the MTHFR C677T transition is higher than in control subjects with the wild-type genotype, it is most likely due to an impairment of activity of the thermolabile enzyme. In this study, all subjects had adequate folate status as assessed by plasma and RBC folate; notably, all blood homocysteine concentrations were normal. Whether the rate of conversion of ingested 5-HCO-H$_4$ folic acid to 5-CH$_3$H$_4$folic acid will differ in homozygotes for the C677T transition with inadequate folate status remains to be determined.

An alternate explanation is that the 5-mg dose was inadequate (i.e., too high or too low) to discriminate between the two MTHFR phenotypes. However, a recent study in our laboratory (Lathrop et al., unpublished data) showed that compared with the wild-type enzyme, the in vitro activity of the T/T MTHFR variant from extracts of lymphocytes remains depressed at a range of added substrate.

It is also possible that the in vivo expression of the T/T MTHFR genotype differs among various tissues. We recently determined, in these same subjects plus seven others, the distribution of folate forms in RBC (Bagley and Sellhau 1998). In individuals with wild-type MTHFR, RBC contained exclusively 5-CH$_3$H$_4$folic acid polyglutamates. However in most individuals with the T/T MTHFR variant, RBC contained, in addition to 5-CH$_3$H$_4$folic acid polyglutamates, formyltetrahydrofolic acid polyglutamates in a proportion that ranged from 0 to 58% of total folates. In all subjects, total RBC folate status remains to be explored.

LITERATURE CITED


