Methylenetetrahydrofolate Reductase 677C→T Variant Modulates Folate Status Response to Controlled Folate Intakes in Young Women1,2

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ABSTRACT A common genetic variant in the methylenetetrahydrofolate reductase (MTHFR) gene involving a cytosine to thymidine (C→T) transition at nucleotide 677 is associated with reduced enzyme activity, altered folate status and potentially higher folate requirements. The objectives of this study were to investigate the effect of the MTHFR 677 T allele on folate status variables in Mexican women (n = 43; 18–45 y) and to assess the adequacy of the 1998 folate U.S. Recommended Dietary Allowance (RDA), 400 μg/d as dietary folate equivalents (DFE). Subjects (14 CC, 12 CT, 17 TT genotypes) consumed a low folate diet (135 μg/d DFE) for 7 wk followed by repletion with 400 μg/d DFE (7 CC, 6 CT, 9 TT) or 800 μg/d DFE (7 CC, 6 CT, 8 TT) for 7 wk. Throughout repletion with 400 μg/d DFE, the TT genotype had lower (P ≤ 0.05) serum folate and higher (P ≤ 0.05) plasma total homocysteine (tHcy) concentrations than the CC genotype. CT heterozygotes did not differ (P > 0.05) in their response relative to the CC genotype. Throughout repletion with 800 μg/d DFE, the CT genotype had lower (P ≤ 0.05) serum folate concentrations and excreted less (P ≤ 0.05) urinary folate than the CC genotype. However, there were no differences (P > 0.05) in the measured variables between the TT and CC genotypes. Repletion with 400 μg/d DFE led to normal blood folate and desirable plasma tHcy concentrations, regardless of MTHFR C677T genotype. Collectively, these data demonstrate that the MTHFR C→T variant modulates folate status response to controlled folate intakes and support the adequacy of the 1998 folate U.S. RDA for all three MTHFR C677T genotypes.


KEY WORDS: • folate • homocysteine • human • MTHFR genotype • RDA

Methylenetetrahydrofolate reductase (MTHFR)4 is an important regulatory enzyme in folate-dependent one-carbon metabolism (Fig. 1). MTHFR catalyzes the irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the major form of folate in plasma and the predominant methyl donor in homocysteine remethylation to methionine. After activation of methionine to S-adenosylmethionine (SAM), the folate-derived methyl group may be used to methylate numerous compounds including DNA. Alternatively, the substrate of MTHFR, 5,10-methylenetetrahydrofolate, may serve as a one-carbon donor in the synthesis of purines and the pyrimidine, thymidine.

The commitment of one-carbon units to methylneogenesis or nucleotide synthesis is controlled by SAM through its effect on MTHFR activity (1–3). Under conditions of low SAM, MTHFR is stimulated to direct the pool of one-carbon units toward methylneogenesis (1–4). In contrast, elevations in SAM inhibit MTHFR activity, thus increasing the availability of one-carbon units for nucleotide synthesis (3). Dietary deficiencies may also affect MTHFR activity directly (i.e., riboflavin) (5,6) or indirectly (i.e., folate, vitamins B-12 and B-6, choline, methionine) by altering the cellular concentrations of methionine-cycle intermediates (1,7). MTHFR activity is also modulated by common single-nucleotide polymorphisms in the MTHFR gene (8–12). The best-characterized MTHFR genetic polymorphism is the cytosine to thymidine (C→T) transition at nucleotide 677, resulting in a codon that encodes valine rather than alanine (8). Individuals possessing one or two T alleles have 30 and 65%, respectively, lower MTHFR activities in vitro than that of the 677 CC genotype (8). The MTHFR 677 TT genotype is associated with reduced folate status (13–15), DNA hypomethylation (16,17) and plasma homocysteine elevations (8), particularly in those with low folate status (15,18,19), and may modulate risk for cardiovascular disease (20–22), cancers (23) and developmental anomalies (24–27).

The MTHFR 677 TT genotype was recognized as a genetic factor that may alter folate requirements during derivation of...
were randomized to consume total folate of 400 or 800 μg/d DFE, respectively. Because of the large number of subjects (n = 43), the feeding phase was conducted in cohorts over 18 mo. To reduce systematic errors (i.e., selection bias, selection/maturity interaction and experimental mortality), all three MTHFR C677T genotypes were enrolled in each cohort.

Breakfast and dinner were consumed daily in the Human Nutrition's metabolic kitchen at Cal Poly Pomona University. Subjects were allowed to consume lunch and snacks as well as 14 meals (breakfast and/or dinner) of their choosing off-site. Weight was monitored weekly and any deviation of ±5% from baseline was addressed by modifying energy intake with folate-free items such as sodas, gelatin, whipped topping and margarine. The principal investigator and/or trained graduate students had daily contact with the subjects to help ensure compliance.

**Diet and supplements**

A low folate 5-d rotation menu (Table 2), consisting of nonfolic acid–fortified foods, was designed. The folate content (mean ± sd) of the diet, as determined by trienzyme methodology on three separate occasions, was 135 ± 15 μg/d. Unfortified flour (Kansas State University, Manhattan, KS) was used to prepare folic acid–fortified foods, normally obtained commercially, including biscuits, waffles, chocolate chip and oatmeal cookies, pizza dough, chocolate cupcakes and blueberry muffins. Chicken, ground beef, carrots and green beans were boiled three times for 10 min each to reduce the amount of folate in these foods, as previously described (30). Menu items were weighed to the nearest 0.5 g to ensure that all subjects received the same amount of food folate.

Energy and all other nutrients in the diet, except for folate, were analyzed by use of the ESHA Food Processor Nutrient Data Base (version 7.81; ESHA Research, Salem, OR). The diet provided a mean energy intake of 8828 kJ/d with ~60, 10 and 30% of the energy from carbohydrate, protein and fat, respectively. Subjects were given supplements to provide the 1998 dietary reference intakes (DRI) for the B vitamins (except folate) and choline, and the 1989 RDA for all other essential nutrients not met by the diet. The supplements included a multimineral given every day (LifeTime, Nutritional Specialties, Anaheim, CA); a multivitamin (Trader Darwin’s Stress Vitamin; Trader Joe’s, South Pasadena, CA), cut into thirds and given every 4th d; vitamin K (KAL, Nutraceutical Corp., Park City, UT), given every other day; choline (TwinLab, Twin Laboratories, Ronkonkoma, NY), given every other day; and iron (TwinLab, Twin Laboratories) given as needed (based on weekly hematocrit measures). The diet and supplements provided ~85% of the 1989 RDA for calcium and 90 to 120% of the 1989 RDA or 1998 DRI for all other essential vitamins (except folate) and minerals. The water-soluble vitamins lost during boiling of food items as well as the forticants normally in enriched flour were adjusted for in the calculations.

Folic acid supplements were prepared from commercially available folic acid (Sigma Chemical, St. Louis, MO) and were consumed at

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**Table 1**

<table>
<thead>
<tr>
<th>Study design</th>
<th>Depletion (wk 0–7)</th>
<th>Repletion 1 (wk 8–14)</th>
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<tbody>
<tr>
<td>MTHFR C677T Genotype</td>
<td>135 μg/d DFE</td>
<td>400 μg/d DFE</td>
</tr>
<tr>
<td>CC</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>CT</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>TT</td>
<td>17</td>
<td>9</td>
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</tbody>
</table>

1 Subjects were randomized to 400 or 800 μg/d dietary folate equivalents (DFE) derived from 135 μg natural food folate plus 156 or 391 μg synthetic folic acid.

2 MTHFR, methylenetetrahydrofolate reductase.
the morning and evening meals throughout repletion, under the supervision of the investigators. To make the folic acid solution for repletion with 400 and 800 µg/d DFE, weighed folic acid was dissolved in a small amount of 0.1 mol/L NaOH and brought to volume with 0.1 mol/L phosphate buffered saline (pH 7.0). The folic acid concentration was determined spectrophotometrically at 282 nm, with a molar absorptivity coefficient of 27,600 L/(mol·cm) (31). The folic acid stock solution was prepared once during the study, dispensed into 200-mL containers, wrapped in aluminum foil and stored at −80°C. Appropriate volumes of the folic acid solution were dispensed into 50-mL conical tubes to which ~40 mL apple juice was added. The tubes were then wrapped in aluminum foil and frozen at −20°C. The folic acid supplements for subject consumption were prepared every 3 mo from the folic acid stock solution. Spectrophotometric analysis demonstrated that losses of folic acid during storage at −80°C were negligible.

Sample collection and blood processing

Baseline and weekly fasting (10 h) venous blood samples were collected in serum separator gel and clot-activator tubes (SST, Vacutainer, Becton Dickinson, Rutherford, NJ) and EDTA tubes (Vacutainer). Serum was collected after centrifugation (450 × g for 15 min at 21°C), dispensed into 1.5-mL microcentrifuge tubes containing 10–15 mg of ascorbic acid and stored at −80°C. Whole blood from the EDTA-containing tube was mixed, dispensed (50 µL) into 1.5-mL microcentrifuge tubes and diluted 1:20 with 1 µL ascorbic acid solution (950 µL). After vortexing, the diluted whole blood was incubated at room temperature for 30 min and stored at −80°C. Hematocrits were also measured by use of mixed EDTA whole blood. The blood was drawn up into two microhematocrit tubes (Fisher Scientific, Pittsburgh, PA), plugged with clay (Crito SEAL; Oxford Labware, St. Louis, MO) and centrifuged. The capillary tubes were then measured on a microcapillary reader (International Equipment, Needham Heights, MA). Plasma and leukocytes were collected from EDTA blood that was immediately placed on ice and centrifuged within 1 h of the blood draw at 1800 × g for 15 min at 4°C. Plasma was dispensed into 1.5-mL microcentrifuge tubes and stored at −80°C for plasma tHcy analyses. For genotyping, the buffy layer representing peripheral leukocytes (~300 µL) was removed, dispensed into 1.5-mL microcentrifuge tubes containing 50 µL dimethyl sulfoxide (DMSO; Sigma Chemical), mixed by inversion and frozen at −80°C.

The 24-h urine collections were obtained at baseline and weekly throughout the study in acid-washed 2-L brown plastic bottles containing 5 g of ascorbate. Subjects were advised to keep urine refrigerated at all times to prevent bacterial growth and folate breakdown. Weekly urine samples from each subject were mixed thoroughly, dispensed into 250-mL containers and stored at −20°C.

Analytical methods

Folate content of diet. The folate content of the diet was determined before starting the study and twice during the study. Each meal including beverage was prepared as for the subject’s consumption, blended with 150 mL of cold 0.1 mol potassium phosphate buffer/L (pH 6.3) containing 57 mmol ascorbic acid/L to preserve the folate (32), dispensed into 50-mL conical tubes and stored at −20°C. Duplicates of the blended samples were thawed, homogenized and subjected to trienzyme treatment (33) and double extraction (34) by use of the Hepes/Ches buffer (pH 7.85) with ascorbate and 2-mercaptoethanol (35). Total food folate was then measured microbiologically (36).

MTHFR C677T genotype. DNA for genotyping was extracted from leukocytes by use of a commercially available kit (QiAamp blood kit; Qiagen, Santa Clarita, CA). Determination of the C677T MTHFR genotype involved polymerase chain reaction (PCR) and HinfI restriction enzyme digestion, as described by Frost and associ-

## TABLE 2

<table>
<thead>
<tr>
<th>D 1</th>
<th>D 2</th>
<th>D 3</th>
<th>D 4</th>
<th>D 5</th>
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<tr>
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<td><strong>Waffle</strong></td>
<td><strong>Granola cereal</strong></td>
<td><strong>Waffle</strong></td>
<td><strong>Granola cereal</strong></td>
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<tr>
<td></td>
<td>Blueberries and whipped topping syrup, margarine</td>
<td>Raisins</td>
<td>Syrup, margarine</td>
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<tr>
<td></td>
<td>Cranapple juice</td>
<td>Mocha mix</td>
<td>Chunky spiced applesauce</td>
<td>Mocha mix</td>
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<td></td>
<td></td>
<td>Applesauce</td>
<td>Cranapple juice</td>
<td>Applesauce</td>
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<td>Ham</td>
<td><strong>Tuna</strong></td>
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<td>Soda</td>
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<td></td>
<td>Soda</td>
<td></td>
<td></td>
<td>Soda</td>
</tr>
<tr>
<td><strong>Dinner</strong></td>
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<td>Chicken enchiladas</td>
<td><strong>Spaghetti</strong></td>
<td>Chicken breast</td>
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<td>Green beans and carrots</td>
<td>Green beans, canned</td>
<td>Green beans and carrots</td>
<td>Barbeque sauce</td>
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<td>Cranapple juice</td>
<td>Orange sherbet</td>
<td>Peach crisp</td>
<td>Biscuit</td>
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<td></td>
<td>Cranapple juice</td>
<td>Cranapple juice</td>
<td>Chocolate cupcake</td>
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<td></td>
<td></td>
<td></td>
<td>Cranapple juice</td>
</tr>
<tr>
<td><strong>Snacks</strong></td>
<td><strong>Popcorn</strong></td>
<td>Apple and cinnamon rice cakes</td>
<td><strong>Popcorn</strong></td>
<td>Apple and cinnamon rice cakes</td>
</tr>
</tbody>
</table>

1 Menus analyzed by trienzyme folate extraction method provided a mean of 135 µg/d dietary folate equivalents (DFE).
2 Menu items prepared with unfortified flour were waffles, blueberry muffins, cookies, chocolate cupcakes, pizza dough and biscuits. Unfortified flour also used in sauces for tuna casserole and chicken enchiladas and for the peach crisp topping.
3 Chicken, hamburger, green beans and carrots were thrice boiled for 10 min each boil to reduce folate content.
4 Foods allowed in unlimited amounts included soda, jello and whipped topping; also two cups of coffee or tea were allowed per day.
ates (8). In the absence of the MTHFR C677T variant, there was a single uncut PCR product of 198 base pairs. In the presence of the MTHFR polymorphism, a HindI restriction site was created, resulting in digestion of the 198-bp fragment into 175 and 23 bp. The PCR products were separated by gel electrophoresis on a 1.5% agarose gel and viewed under UV light.

**Blood and urinary folate.** Folate concentrations of serum, erythrocytes and urine were determined microbiologically by use of the microtitre plate adaptation with *Lactobacillus casei* (36). The intra- and interassay CV were both 12%, based on the positive control.

### Statistical analysis

All data summarization and analyses were performed by use of SPSS10.0 for Windows (SPSS, Chicago, IL). To determine whether baseline differences existed between the MTHFR C677T genotypes in the measured variables (serum folate, red cell folate, urinary folate and plasma tHcy), one-way ANOVA was used. Where a significant genotype effect was detected by ANOVA, the Tukey HSD test was used for mean separation.

The relationship between the folate status response variable and MTHFR C677T genotypes throughout the depletion phase was examined by use of the repeated-measures ANOVA facility of the GLM procedure with one within-factor (weeks) variable and one between-factor (genotype) variable. For the repletion phase of the study, a repeated-measures ANOVA with one within-factor (weeks) variable and one between-factor (genotype and folate intake) variables was used to examine the interaction effects. Because there were no significant three-way interactions, the data were stratified by folate intake, 400 and 800 μg/d DFE, and the relationships between the folate status response variables and MTHFR C677T genotypes throughout the repletion phase were examined by use of a repeated-measures ANOVA with one within-factor (weeks) variable and two between-factor (genotype and folate intake) variables was used to examine the interaction effects. Because there were no significant three-way interactions, the data were stratified by folate intake, 400 and 800 μg/d DFE, and the relationships between the folate status response variables and MTHFR C677T genotypes throughout the repletion phase were examined by use of the repeated-measures ANOVA with one within-factor (weeks) variable and one between-factor (genotype) variable. Significant genotype effects were examined by use of the Tukey HSD test and significant week effects were examined by use of simple and repeated contrasts. Where a significant Mauchly’s test of sphericity was obtained, the Greenhouse–Geisser epsilon was applied to protect against a type 1 error. Missing values from the red cell folate data (n = 4) were calculated by use of weighted averages on the basis of subject, genotype, folate intake and week grouping. One subject’s urinary folate data were excluded from the repletion phase because of incomplete collections. Significance was set at P ≤ 0.05. Data are presented as means ± SD in the text and tables and means ± SEM in the figures.

### RESULTS

#### Subject characteristics and baseline measures

The final study group was composed of 43 women with the following MTHFR C677T genotype distribution: 14 wild type (CC), 12 heterozygous (CT), and 17 homozygous for the T variant (TT). The mean age of the women was 25 y (range = 18–44 y), whereas the mean body mass index (kg/m²) was 25.2 (range = 19.5–32). No differences (P > 0.05) were detected in age or body weight between women representing the three MTHFR C677T genotypes at the beginning or end of study. Body weights were maintained within 5% of baseline in all but nine subjects (3 CC, 3 CT and 3 TT) who lost ~8% (range: 6.5–10.7%) of baseline weight. A total of 11 women (4 CC, 3 CT and 4 TT) reported using oral contraceptives during the study period. No differences (one-way ANOVA; P > 0.05) existed in serum folate, urinary folate or homocysteine concentrations between the MTHFR genotypes at baseline (Table 3). Red cell folate concentrations were lower (P ≤ 0.05) in the TT genotype than in the CC control, although CT and CC genotypes did not differ (P > 0.05) (Table 3).

#### Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF, nmol/L</td>
<td>33.9 ± 13.5</td>
<td>28.6 ± 10.1</td>
<td>29.8 ± 9.4</td>
</tr>
<tr>
<td>RCF, nmol/L</td>
<td>1202 ± 188a</td>
<td>1087 ± 190ab</td>
<td>993 ± 258b</td>
</tr>
<tr>
<td>UF, nmol/d</td>
<td>46.4 ± 40</td>
<td>29.8 ± 30</td>
<td>70.5 ± 121</td>
</tr>
<tr>
<td>tHcy, μmol/L</td>
<td>5.3 ± 0.9</td>
<td>5.9 ± 0.9</td>
<td>5.4 ± 0.6</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 12–17.
2 Means in a row with superscripts without a common letter differ, P ≤ 0.05.

#### Serum folate

Folate depletion decreased (P ≤ 0.05) the serum folate concentration 58%, from 38.8 ± 11.0 to 12.9 ± 4.9 nmol/L. Subjects possessing the TT genotype had lower (P ≤ 0.05) serum folate concentrations throughout depletion compared to the CC controls, whereas those with the CT genotype were numerically lower (P = 0.13; Fig. 2A).

Folate repletion with 400 μg/d DFE increased (P ≤ 0.05) the serum folate concentration 23%, from 13.9 ± 5.3 to 17.1 ± 5.2 nmol/L. However, baseline serum folate concentrations were not obtained and 32% had serum folate concentrations in the low normal range, 6.8–13.6 nmol/L. Throughout repletion with 400 μg/d DFE, subjects possessing the TT genotype had lower (P ≤ 0.05) serum folate concentrations than either the CC or CT genotypes (Fig. 2B). At the end of repletion with 400 μg/d DFE, 50, 28.5 and 14% of the TT, CT and CC genotypes, respectively, had serum folate concentrations in the low normal range. No subjects had serum folate concentrations < 6.8 nmol/L.

Folate repletion with 800 μg/d DFE increased (P ≤ 0.05) the serum folate concentration 223%, from 11.9 ± 4.4 to 38.4 ± 9.6 nmol/L, which was greater (P ≤ 0.05) than baseline concentrations. A genotype effect was evident during repletion with 800 μg/d DFE, with the CT genotype exhibiting lower (P ≤ 0.05) serum folate concentrations than the CC controls (Fig. 2C). The response of the TT genotype was intermediate.

During the repletion phase of the study, there were no three-way or genotype/week interactions for serum folate. However, a significant (P ≤ 0.01) folate intake/week interaction was detected. In the 400 μg/d DFE group, serum folate increased (P ≤ 0.01) modestly during the 1st wk of repletion and remained stable, whereas for the 800 μg/d DFE group, serum folate concentrations doubled during the 1st wk and continued to increase (P ≤ 0.05) until wk 11 (Fig. 2B, C); furthermore, there was a significant (P ≤ 0.05) genotype/folate intake interaction. The genotype response pattern for serum folate to 400 μg/d DFE was CC > CT > TT compared to CC > TT > CT for 800 μg/d DFE.

#### Red cell folate

Folate depletion decreased (P ≤ 0.05) the red cell folate concentration 25%, from 1088 ± 231 to 820 ± 163 nmol/L. Throughout depletion, subjects possessing the TT genotype had lower (P ≤ 0.05) red cell folate concentrations than those
interaction. In the 400 μg/d DFE group, red cell folate declined slightly and then remained stable at postdepletion levels. For the 800 μg/d DFE group, red cell folate concentrations increased by wk 10 (P ≤ 0.05) and remained higher (P ≤ 0.01) than postdepletion concentrations throughout the study (Fig. 3B, C).

**Urinary folate**

Folate depletion decreased (P ≤ 0.05) the urinary folate excretion 79%, from 51.2 ± 81.4 to 10.9 ± 4.8 nmol/d. Genotype did not affect urinary folate response (Fig. 4A).

After repletion with 400 μg/d DFE, urinary folate excretion increased, but not significantly, 20%, from 11.3 ± 5.8 to 13.6 ± 5.1 nmol/d. Baseline concentrations were not achieved and no genotype effect was detected (Fig. 4B).

Repletion with 800 μg/d DFE increased (P ≤ 0.05) the urinary folate excretion 47%, from 9.9 ± 6.0 to 14.3 ± 7.1 nmol/d. Genotype did not affect urinary folate response (Fig. 4C).

![FIGURE 3](https://academic.oup.com/jn/article-abstract/133/5/1272/4616755) Weekly red cell folate concentrations in Mexican American women with differing MTHFR C677T genotypes during folate depletion (14 CC, 12 CT, 17 TT) (A) and repletion with 400 μg/d DFE (7 CC, 6 CT, 9 TT) (B) and 800 μg/d DFE (7 CC, 6 CT, 8 TT) (C). *, **Weekly red cell folate concentration for the combined MTHFR genotypes (n = 43) differs from wk 0 (A) or wk 7 (B, C) at P = 0.05 or P = 0.01, respectively. †, †† Weekly red cell folate concentration for the combined MTHFR genotypes (n = 43) differs from the preceding week at P = 0.05 or P = 0.01, respectively. Values are means ± SEM. a, b MTHFR genotypes with differing superscript letters differ across all weeks, P ≤ 0.05.
urinary folate excretion 294%, from 10.5 ± 3.7 to 42.9 ± 29.1 nmol/d, which was similar (P = 0.05) to baseline urinary folate concentrations. Throughout repletion with 800 µg/d DFE, the CT genotype excreted less (P = 0.05) urinary folate than the CC controls (Fig. 4C). The urinary folate excretion of the TT genotype also tended to be lower (P = 0.06) than the CC controls.

During the repletion phase of the study, there were no three-way or genotype/week interactions for urinary folate. However, a significant (P ≤ 0.01) folate intake/week interaction was detected. In the 400 µg/d DFE group, urinary folate excretion did not change (P > 0.05) from postdepletion concentrations throughout the repletion phase. For the 800 µg/d DFE group, urinary folate excretion increased (P ≤ 0.05) by wk 8 and again at wk 9 before stabilizing (Fig. 4B, C). Moreover, there was a significant (P ≤ 0.05) genotype/folate intake interaction. The genotype response pattern to 400 µg/d DFE for urinary folate was CC > CT > TT compared to CC > TT > CT for 800 µg/d DFE.

**Homocysteine**

Folate depletion increased (P ≤ 0.05) the plasma tHcy concentration 31%, from 5.5 ± 0.83 to 7.2 ± 1.2 µmol/L. Throughout depletion, tHcy concentrations between the MTHFR C677T genotypes did not differ (P > 0.05) (Fig. 5A).

Repletion with 400 µg/d DFE decreased (P ≤ 0.05) the tHcy concentration 22%, from 7.1 ± 1.2 to 5.6 ± 0.93 µmol/L, and all subjects had plasma tHcy concentrations in the desirable range (i.e., <10 µmol/L). Throughout repletion with 400 µg/d DFE, the TT genotype had higher (P ≤ 0.05) tHcy concentrations than that of the CC control but the CT and CC genotypes did not differ (P > 0.05) (Fig. 5B).

Repletion with 800 µg/d DFE decreased (P ≤ 0.05) the plasma tHcy concentration 24%, from 7.4 ± 1.2 to 5.5 ± 0.81 µmol/L.
μmol/L. Throughout repletion with 800 μg/d DFE, the MTHFR C677T genotypes did not differ (P > 0.05) (Fig. 5C).

During the repletion phase of the study, there were no three-way or genotype/week interactions for plasma tHcy; however, there was a significant (P ≤ 0.05) folate intake/week interaction. In the 400 μg/d DFE group, plasma tHcy decreased (P ≤ 0.01) initially at wk 9 but increased (P ≤ 0.01) to postdepletion concentrations at wk 11 and 13. In the 800 μg/d DFE group, the initial decline (P ≤ 0.01) in plasma tHcy concentrations at wk 8 was maintained throughout the repletion phase (Fig. 5B, C); in addition there was a significant (P ≤ 0.05) genotype/folate intake interaction. The genotype response pattern to 400 μg/d DFE for plasma tHcy was CC < CT < TT compared to TT < CC < CT for 800 μg/d DFE.

**DISCUSSION**

Data derived from observational and intervention studies suggest that the MTHFR C→T polymorphism negatively influences folate and homocysteine status (8,13,15,19,39–41). We designed the present study to test the hypothesis that the MTHFR 677 C→T variant modulates folate status and to assess the adequacy of the 1998 folate U.S. RDA. Unique aspects of the study design included strict control of folate intake; repletion with the 1998 folate U.S. RDA, 400 μg/d DFE, and a higher level, 800 μg/d DFE, after a moderate folate depletion phase; and inclusion of premenopausal women of Mexican descent representing all three MTHFR C677T genotypes.

The results of this study provide definitive data that the MTHFR C→T polymorphism negatively influences folate and homocysteine status responses. Throughout folate depletion, the TT genotype had lower (P ≤ 0.05) serum folate and red cell folate concentrations compared to those of the CC control. Throughout repletion with 400 μg/d DFE, the TT genotype had lower (P ≤ 0.05) serum folate concentrations and higher (P ≤ 0.05) plasma tHcy concentrations than those of the CC control. During repletion with 800 μg/d DFE, the CT genotype had lower (P ≤ 0.05) serum folate concentrations and excreted less (P ≤ .05) urinary folate than those of the CC control.

The lower (P ≤ 0.05) folate status observed in the TT genotype relative to the CC genotype was confined to those consuming 400 μg/d DFE or less. On the highest folate intake, 800 μg/d DFE, no differences (P > 0.05) in folate status were observed between the TT and CC genotypes. These data are consistent with previous studies that have demonstrated an interaction between folate status/intake and the MTHFR 677 TT genotype (18,19,39). In the present study, the genotype response on 400 μg/d DFE followed the expected pattern, TT < CT < CC for serum and urinary folate and TT > CT > CC for plasma tHcy concentrations. A different (P ≤ 0.05) pattern emerged, however, between the MTHFR C677T genotypes repleted with 800 μg/d DFE, CT < TT < CC for serum and urinary folate and CT > CC > TT for plasma tHcy. The data regarding plasma tHcy, however, must be interpreted with caution since plasma tHcy concentrations for the TT genotype were numerically the highest and lowest at the beginning of the repletion phase with 400 and 800 μg/d DFE, respectively. Possible explanations for the apparent enhanced sensitivity of the 677 TT genotype to increased folate intake observed in the present study and others (15,18,39,40,42,43) may include decreased loss of the MTHFR flavin cofactor in the presence of high folate (44), upregulation of MTHFR and/or upregulation of hepatic betaine:homocysteine methyltransferase (45).

The relatively low plasma tHcy concentrations in all three MTHFR C677T variants after folate depletion (7.2 μmol/L), although unexpected, may be attributable to the low initial tHcy concentrations (5.5 μmol/L). Other studies have reported an ~2 μmol/L increase in plasma tHcy after a low folate diet (15,46), which is similar to the increase in the present study. Race or ethnicity may also be a factor because lower plasma tHcy concentrations have been reported in persons of Mexican descent compared to Caucasians (47).

Sufficient dietary intake of the lipotropes, methionine and choline, as well as the B vitamins that serve as cofactors for homocysteine metabolizing enzymes (i.e., riboflavin, vitamins B-12 and B-6), also provide a plausible explanation for the lack of hyperhomocysteinemic individuals after low folate intake. Choline, for example, is utilized as a methyl donor when folate intake is low (48).

When establishing the 1998 folate U.S. RDA, 400 μg/d DFE, red cell folate concentration was used as the primary indicator of folate status, whereas serum folate and plasma tHcy concentrations served as ancillary markers (28). Red cell folate concentration reflects tissue stores and is considered a long-term indicator of folate status (49,50). Serum folate reflects recent dietary folate intake (50); however, under conditions of controlled folate intake, repeated measurements of serum folate concentrations in the same person over time reflect changes in folate status (28). Plasma tHcy is a sensitive functional indicator of folate status and increases under conditions of folate inadequacy (30,32,46). However, plasma tHcy is a nonspecific marker of folate status, because other vitamin deficiencies, genetic variations and exogenous factors may influence it (51,52). In the present study, repletion with 400 μg/d DFE achieved or maintained serum folate concentrations > 6.8 nmol/L, red cell folate concentrations > 317 nmol/L and plasma tHcy concentrations < 12 μmol/L, concentrations reported by the IOM 1998 (28) to reflect folate sufficiency. However, 50% of women possessing the 677 TT genotype and 32% overall had serum folate concentrations indicative of marginal deficiency (between 6.8 and 14 nmol/L); the end of the repletion period with 400 μg/d DFE. This may be physiologically unimportant because plasma tHcy concentrations were low (5.6 ± 0.9 μmol/L) after folate repletion with 400 μg/d DFE and similar (P > 0.05) to plasma tHcy concentrations in the 800 μg/d DFE group, regardless of MTHFR C677T genotype. Together, these data suggest that the 1998 folate U.S. RDA is sufficient for all three MTHFR C677T genotypes.

The role of folate in reducing the risk of vascular disease, cancer and psychiatric mental disorders was also considered when devising the 1998 folate U.S. RDA. Data, however, were not sufficient to serve as a basis for setting folate recommendations (28). Of the folate status indicators, the functional indicator, plasma tHcy, has garnered the most attention and is considered a risk factor for vascular disease when elevated (20). In the present study, the 1998 folate U.S. RDA was effective in preventing even mild elevations in plasma tHcy concentrations in all subjects, regardless of MTHFR C677T genotype, and will provide useful information if future folate U.S. RDAs are based on risk reduction of chronic diseases or developmental disorders.

To summarize, these data demonstrate that the MTHFR C→T variant modulates folate status response to controlled folate intakes. The lack of relationship between the TT genotype and folate nutriture at the highest intake, 800 μg/d DFE, indicates that folate intake plays a critical role in the ultimate determination of phenotype. Importantly, the 1998 folate U.S. RDA, 400 μg/d DFE, led to normal blood folate concentrations and desirable plasma tHcy concentrations in women of Mexican descent, regardless of MTHFR C677T genotype.


