5,10-Methylentetrahydrofolate reductase genotype determines the plasma homocysteine-lowering effect of supplementation with 5-methyltetrahydrofolate or folic acid in healthy young women1–3

Iris P Fohr, Reinhild Prinz-Langenohl, Anja Brönstrup, Anja M Bohlmann, Heinz Nau, Heiner K Berthold, and Klaus Pietrzik

ABSTRACT
Background: Elevated plasma total homocysteine (tHcy) is a risk factor for vascular disease and neural tube defects. The polymorphism in the gene encoding 5,10-methylentetrahydrofolate reductase (FADH2) (MTHFR) influences the tHcy concentration and the response to tHcy-lowering therapy. Supplementation with folic acid (FA) decreases plasma tHcy, but limited data are available on the effect of 5-methyltetrahydrofolate (MTHF).

Objective: We evaluated the tHcy-lowering potential of low-dose FA and of MTHF with respect to the MTHFR genotype.

Design: In this randomized, placebo-controlled, double-blind study, 160 women received 400 μg FA, the equimolar amount of MTHF (480 μg, racemic mixture), or a placebo daily during an 8-wk treatment period. Blood samples were collected at baseline and at 4 and 8 wk.

Results: Changes in plasma tHcy concentration depended on the supplemented folate derivative and the MTHFR genotype. Supplementation with FA significantly decreased tHcy concentrations by ≥13% in women of all 3 genotypes after both 4 and 8 wk. The greatest decrease was 20% (P < 0.05) in the women with the TT genotype after 4 wk. MTHF supplementation also decreased tHcy, but only the women with the CT genotype had a significant decrease after 4 wk (7%; P < 0.05). The largest non-significant reduction (15%) occurred in the women with the TT genotype after 4 wk of MTHF supplementation.

Conclusions: The response to tHcy-lowering therapy is influenced by MTHFR genotype. Women with the TT genotype seem to benefit the most from supplementation with either FA or MTHF. In women with the CT or CC genotype, FA is more effective than MTHF in lowering plasma tHcy. Am J Clin Nutr 2002;75:275–82.

KEY WORDS  MTHFR genotype, homocysteine, folic acid, methyltetrahydrofolate, methylenetetrahydrofolate reductase, supplementation, women

INTRODUCTION
Homocysteine is widely accepted as an independent risk factor for vascular disease (1–3) and fetal malformations such as neural tube defects (NTDs) (4, 5). Evidence from epidemiologic studies also suggests a role for homocysteine in other pregnancy complications like placental abruption, preeclampsia, and spontaneous abortion (6, 7). In addition, plasma homocysteine is considered a sensitive marker of folate status (8–10).

Elevated plasma homocysteine concentrations have been linked both to the inadequate status of vitamin cofactors (ie, folate, vitamin B-12, and vitamin B-6) and to genetic defects in enzymes involved in homocysteine metabolism. Consequently, hyperhomocysteinemia can be considered a result of a gene-nutrient interaction (11, 12). The most common gene defect is the 677C→T mutation in the gene for 5,10-methylentetrahydrofolate reductase (FADH2) (MTHFR); this mutation causes a variant of the enzyme that is thermolabile and has reduced activity (13). MTHFR catalyzes the formation of 5-methyltetrahydrofolate (MTHF), the biologically active folate form needed for homocysteine degradation in the remethylation pathway. Impaired methylation of homocysteine increases plasma total homocysteine (tHcy) concentrations (14, 15). Among persons with poor folate status, those homozygous for the 677C→T mutation (TT genotype) express higher tHcy concentrations than do persons having the CC genotype (12, 16). Many reports, although not all (17), indicate that the TT genotype is related to certain forms of vascular disease. In addition, the MTHFR polymorphism is identified as a genetic risk factor for NTDs (18–20). This agrees with the observed involvement of maternal hyperhomocysteinemia in the etiology of NTDs (4, 5).

Folic acid (pteroylmonoglutamic acid; FA) supplementation is considered to be the treatment of choice for lowering plasma tHcy concentrations. Doses of ∼400 μg FA/d have been shown to be effective in several studies (21–26). Authorities recommend the intake of 400 μg FA/d in the periconceptional period to prevent the occurrence of NTDs (27, 28). This dose of FA, however, may

1 From the Department of Pathophysiology of Human Nutrition, Institute of Nutritional Science (IPF, RP-L, AB, and KP), and the Institute of Clinical Pharmacology, Medical School (HKB), University of Bonn, Germany, and the Department of Food Toxicology, Center for Food Sciences, School of Veterinary Medicine, University of Hannover, Germany (AMB and HN).

2 Knoll-BioResearch, S Antonino, Switzerland, kindly supplied the 5-methyltetrahydrofolate as a crystalline powder.

3 Address reprint requests to K Pietrzik, the Department of Pathophysiology of Human Nutrition, Institute of Nutritional Science, University of Bonn, Endenicher Allee 11-13, D-53115 Bonn, Germany, E-mail: k.pietrzik@uni-bonn.de.

Received October 24, 2000.
Accepted for publication March 13, 2001.

result in the appearance of unmetabolized FA in serum (29), which is hypothesized to mask the hematologic manifestations of unrecognized vitamin B-12 deficiency, thereby predisposing persons to irreversible neurologic damage.

Earlier studies usually used FA for supplementation, but this folate derivative itself has to be converted into MTHF to become biologically active. The effect of MTHF supplementation on tHcy concentrations has not been intensively investigated. In 3 recent studies (30–32), supraphysiologic doses were given to patients with hyperhomocysteinemia. The present study was conducted to evaluate the effect of orally administered, low-dose supplementation with either FA or MTHF on plasma tHcy concentrations in healthy women of childbearing age with respect to their MTHFR genotype. An additional goal of the study was to investigate whether supplementation with MTHF improves the tHcy-lowering efficiency in women with the TT genotype relative to FA supplementation.

SUBJECTS AND METHODS

Subjects

Participants were healthy, nonpregnant female volunteers aged 19–39 y. The inclusion criteria were as follows: no recent history of an organic or mental disease, normal results on routine laboratory tests, and an adequate vitamin B-12 status (plasma vitamin B-12 ≥ 110 pmol/L) (33). The main exclusion criteria were abuse of alcohol or drugs, medical treatment interfering with folate metabolism, and pregnancy or lactation. All subjects were asked to maintain their usual dietary habits for the duration of the study but to abstain from the intake of vitamin supplements. The remaining 160 participants were included in the study protocol (either because of an intake of drugs interacting with folate metabolism, or because of a change of residence or personal reasons, and 3 participants withdrew because they failed to adhere to the study protocol (either because of an intake of drugs interacting with folate metabolism or because of a change in permanent medication). The remaining 160 participants were included in the statistical analysis. The study protocol was approved by the Ethical Committee of the Medical School, University of Bonn, and each subject gave written, informed consent.

Study design

In this randomized, placebo-controlled, double-blind study, a 4-wk washout period preceded the 8-wk treatment period. Fast- ing blood samples were collected at the beginning of the washout period (week –4) to determine the health status, vitamin B-12 status, and MTHFR genotype of the participants; samples were also collected at the start of the treatment (week 0) and at 4 and 8 wk. Stratified by genotype, the participants were allocated at random to 1 of the 3 treatment groups: FA (400 µg FA/d), MTHF (480 µg MTHF/d), and placebo. Stratification ensured an equal distribution of the 3 genotype variants within the 3 treatment groups. The vitamin capsules were prepared by PCI Allpack (Schorndorf, Germany) with FA (Synopharm, Barsbüttel, Germany) or the equimolar amount of MTHF (sodium salt, racemic mixture containing 50% each of the 6S and 6R isomers) (Knoll-BioResearch, S Antonino, Switzerland). According to the manufacturer, MTHF is stable. During the 8-wk treatment period, subjects were required to take one capsule every morning before breakfast except on blood collection days, when the capsule was taken after venipuncture. Compliance with the treatment was assessed by pill counting. At each visit to the research center for blood collection, the subjects’ adherence to the study protocol was checked with the use of a questionnaire.

At weeks 0, 4, and 8, venous blood was collected from all participants, who had fasted overnight, into tubes containing EDTA or heparin as an anticoagulant (S-Monovette; Sarstedt, Nümbrecht, Germany). Plasma tHcy and plasma folate were measured at each time point, red blood cell (RBC) folate was measured at weeks 0 and 8, and vitamin B-12 and vitamin B-6 (as pyridoxal-P, PLP) were measured at baseline (week 0). In a subset of 20 participants, the distribution of plasma folate metabolites was also analyzed at weeks 0 and 8.

The blood for measurement of tHcy, plasma folate, and folate metabolites in plasma was immediately cooled on ice and centrifuged within 15 min at 2000 X g for 10 min at 4°C. For all other variables, blood was kept cool (4°C) in the dark before centrifugation or hemolysis. All specimens were stored at −20°C until analysis within 4 mo after completion of the treatment period. Routine laboratory variables were measured immediately after venipuncture at weeks −4 and 8 to evaluate the health status of the participants.

Laboratory investigations

tHcy concentrations in EDTA-treated plasma were measured with the use of reversed-phase HPLC with fluorescence detection according to the method of Araki and Sako (36) and Vester and Rasmussen (37) with minor modifications (intraassay CV: <3.6%; interassay CV: <5.1%). Folate and vitamin B-12 were measured in EDTA-treated plasma with the use of commercially available immunoassay kits for the IMx Analyzer (Abbott, Wiesbaden, Germany); the same kit was used to measure both plasma folate (intraassay CV: <2.9%; interassay CV: <4.8%) and RBC folate (intraassay CV: <7.4%; interassay CV: <7.3%). Plasma folate was measured after manually diluting all samples with the IMx folate specimen diluent (1:2). The RBC hemo- lysate was prepared by incubating whole blood with IMx folate lysis reagent in the dark at room temperature for 30 min before freezing the mixture. The folate pattern in heparin-treated plasma was determined according to the method of Belz and Nau (38) with the use of HPLC coupled with a microbiological assay with minor modifications. With this method, the different folate metabolites in plasma are separated with the use of HPLC and quantified with the use of the Lactobacillus casei assay. The HPLC method enables measurement of the different folate metabolites individually, whereas the immunoassay kit measures the sum of all metabolites in plasma. Vitamin B-6 was measured as PLP with the use of HPLC (39, 40). All of the samples from each participant were analyzed within one run to minimize measurement error. The samples for tHcy, distribution of plasma folate metabolites, and PLP were analyzed in duplicate. For 677C→T MTHFR genotyping, DNA was extracted from the leukocyte-rich buffy coat fraction of centrifuged, EDTA-treated whole blood by using the QIAprime DNA blood mini kit (Qiagen, Hilden, Germany); the polymerase chain reaction was performed according to the method of Fross et al (14). All other...
blood variables were measured with the use of standard automated laboratory techniques.

**Statistical analysis**

Because the distributions of the variables tHcy, plasma and RBC folate, vitamin B-12, and PLP were positively skewed initially, logarithmic transformation was carried out to normalize the distributions. The natural logarithms of these variables were used in all statistical analyses as continuous variables. The values of these variables are reported as arithmetic means ± SDs, but geometric means are given for the transformed variables. The baseline concentrations of plasma tHcy, plasma and RBC folate, vitamin B-12, and PLP in the treatment groups were compared by using one-way analysis of variance (ANOVA). The Kruskal-Wallis test was used to compare the variables age, plasma creatinine, and body mass index. Chi-square tests were carried out on categorical variables such as smoking and use of oral contraceptives, prevalence of smoking, and serum creatinine. The serum creatinine concentration, measured as a marker of renal function, was within the normal range. No significant differences between groups were observed in concentrations of tHcy and vitamin cofactors. All but 2 subjects were normohomocysteinemic, with plasma concentrations ranging from 4.4 to 14.0 μmol/L. Two women (one in the FA group and one in the MTHF group with genotype TT) had moderate hyperhomocysteinemia (41), with values of 22.1 and 21.1 μmol/L, respectively.

Thirteen percent of the participants were homozygous for the 677C→T mutation in the MTHFR gene (TT), 47% were heterozygous (CT), and 40% were of the wild type (CC) (Table 2). The tHcy concentration did not differ significantly between the 3 genotype groups, but the subjects with the TT genotype had significantly lower plasma folate concentrations than did the subjects with the CT or CC genotype.

Pill counting indicated good compliance, defined as taking ≤2 capsules more or less than the number permitted during the

### Table 2

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>TT (n = 21)</th>
<th>CT (n = 75)</th>
<th>CC (n = 64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma tHcy (μmol/L)</td>
<td>8.47 ± 3.52 (7.96)</td>
<td>8.50 ± 2.48 (8.21)</td>
<td>8.51 ± 1.86 (8.32)</td>
</tr>
<tr>
<td>Plasma folate (nmoL/L)</td>
<td>12.1 ± 6.1 (10.9)</td>
<td>16.1 ± 6.7 (14.9)</td>
<td>16.4 ± 6.3 (15.5)</td>
</tr>
<tr>
<td>Red blood cell folate (nmoL/L)</td>
<td>353 ± 162 (325)</td>
<td>405 ± 161 (378)</td>
<td>397 ± 125 (381)</td>
</tr>
</tbody>
</table>

/3 ± SD; geometric mean in parentheses.

/Significantly different from the CT and CC genotypes, P < 0.001 (one-way ANOVA with Tukey’s post hoc test).

/Data from 2 participants are missing (1 in the TT group and 1 in the CC group).
8 wk, with an average compliance of 91% (86%, 92%, and 95% in the FA, MTHF, and placebo groups, respectively).

Both supplements, FA and MTHF, were well tolerated and safe: no treatment-related adverse side effects were observed. Values of the investigated clinical chemistry or hematologic blood variables before and after treatment were generally within the respective reference range, although some values deviated from the range slightly. However, these deviations were not clinically relevant.

**Total homocysteine**

Two-way ANOVA revealed a significant interaction between the 2 main effects, treatment group and genotype ($P < 0.05$). As a consequence, the treatment group and the genotype were considered only in combination. The response of plasma tHcy to supplementation in the resulting 9 subgroups is shown in Table 3. The effect of the 3 treatments was different in all 3 genotype groups. Supplementation with FA for 4 wk significantly reduced the geometric mean plasma tHcy concentration in women with the $TT$ genotype by 20% (corresponding geometric mean ratio: 0.80) compared with the baseline concentration. In women with the $CT$ genotype, supplementation with FA significantly reduced mean plasma tHcy concentrations after 4 wk (15%). However, the additional 4 wk of supplementation did not further reduce tHcy concentrations (14% after 8 wk). In contrast, the mean tHcy concentration in subjects with the $CC$ genotype was reduced significantly (13%) only after 8 wk of FA supplementation. Supplementation with MTHF significantly reduced the mean plasma tHcy concentration only in heterozygotes (7%) after 4 wk; none of the changes in the 3 genotype groups after 8 wk were significant.

In the placebo group, the mean plasma tHcy concentration in women with the $CT$ or $CC$ genotype remained unchanged, whereas it increased continuously during the 8-wk treatment period in women with the $TT$ genotype. At the end of the study, the geometric mean tHcy concentration was significantly increased (18%) compared with the baseline concentration.

The most pronounced tHcy-lowering effect occurred in women with the $TT$ genotype. This group showed a significant reduction in plasma tHcy concentration of 20% after 4 wk of supplementation with FA and a nonsignificant reduction of 15% after 4 wk of supplementation with MTHF.

A comparison of the difference in the tHcy-lowering effect between the supplementation strategies within the 3 genotype groups showed that the effect of FA and MTHF in the $TT$ genotype was significantly different from that of placebo after 4 wk. After 8 wk, only the difference between MTHF and placebo was significant ($P < 0.05$). Within the $CT$ genotype group, the difference in the tHcy-lowering effect of FA and placebo was significant after 4 wk ($P < 0.05$), whereas the effect of FA was significantly different from both MTHF and placebo ($P < 0.001$) after 8 wk. In contrast, within the $CC$ group, there were no significant differences between treatment groups after 4 wk, but the difference in the mean tHcy reduction between FA and placebo was significant after 8 wk ($P < 0.05$, one-way ANOVA stratified for genotype with Tukey’s post hoc test).

**Folate**

There was no significant interaction between the main effects, treatment group and genotype, but the treatment influenced the plasma folate concentration significantly ($P < 0.001$, two-way ANOVA). Therefore, results are presented by treatment group only (Table 4). After 4 and 8 wk of vitamin supplementation, the 2 vitamin groups showed significant increases in mean plasma folate concentration, whereas no significant changes were observed in the placebo group. The extent of the mean increase varied according to the supplemented form of folate and the duration of treatment. The geometric mean plasma folate concentration increased by 65% and 86% after 4 and 8 wk, respectively, in the FA group and by 362% and 431% after 4 and 8 wk, respectively, in the MTHF group compared with the baseline concentrations. The difference in the change in plasma folate concentration between all 3 treatment groups was significant ($P < 0.001$; two-way ANOVA).

To investigate the distribution of plasma folate metabolites before and after 8 wk of FA or MTHF supplementation, plasma folate concentrations were measured with the use of HPLC cou-

### Table 3

Response of plasma total homocysteine to supplementation with folic acid (FA) and 5-methyltetrahydrofolate (MTHF) with respect to $MTHFR$ genotype.

<table>
<thead>
<tr>
<th></th>
<th>Week 0</th>
<th>Week 4</th>
<th>Week 8</th>
<th>Mean ratio</th>
<th>Week 4:0</th>
<th>Week 8:0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FA group ($n=51$)</strong></td>
<td>8.50 ± 2.71 (8.19)</td>
<td>7.43 ± 2.21 (7.17)$^4$</td>
<td>7.32 ± 1.73 (7.12)$^4$</td>
<td>0.88</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>$TT$ ($n=5$)</td>
<td>7.37 ± 1.44 (7.27)</td>
<td>5.89 ± 0.98 (5.83)$^4$</td>
<td>6.83 ± 0.92 (6.78)</td>
<td>0.80</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>$CT$ ($n=25$)</td>
<td>8.48 ± 3.23 (8.10)</td>
<td>7.09 ± 1.86 (6.91)$^4$</td>
<td>7.10 ± 1.67 (6.94)$^4$</td>
<td>0.85</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>$CC$ ($n=21$)</td>
<td>8.80 ± 2.24 (8.53)</td>
<td>8.21 ± 2.55 (7.87)</td>
<td>7.69 ± 1.92 (7.44)$^4$</td>
<td>0.92</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td><strong>MTHF group ($n=52$)</strong></td>
<td>8.88 ± 2.75 (8.52)</td>
<td>8.17 ± 1.85 (7.97)$^4$</td>
<td>8.51 ± 2.20 (8.25)</td>
<td>0.93</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>$TT$ ($n=7$)</td>
<td>10.40 ± 5.30 (9.40)</td>
<td>8.25 ± 2.25 (7.96)</td>
<td>8.71 ± 2.91 (8.30)</td>
<td>0.85</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>$CT$ ($n=23$)</td>
<td>8.91 ± 2.49 (8.57)</td>
<td>8.27 ± 2.13 (8.00)$^4$</td>
<td>8.91 ± 2.21 (8.67)</td>
<td>0.93</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td>$CC$ ($n=22$)</td>
<td>8.37 ± 1.67 (8.22)</td>
<td>8.05 ± 1.43 (7.93)</td>
<td>8.02 ± 1.94 (7.83)</td>
<td>0.96</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td><strong>Placebo group ($n=57$)</strong></td>
<td>8.15 ± 1.66 (7.98)</td>
<td>8.09 ± 1.67$^2$ (7.93)</td>
<td>8.51 ± 1.63 (8.36)$^4$</td>
<td>1.00</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>$TT$ ($n=9$)</td>
<td>7.58 ± 2.01 (7.36)</td>
<td>8.37 ± 1.98 (8.14)</td>
<td>8.92 ± 2.00 (8.70)$^4$</td>
<td>1.11</td>
<td>1.18</td>
<td></td>
</tr>
<tr>
<td>$CT$ ($n=27$)</td>
<td>8.06 ± 1.46 (7.93)</td>
<td>7.77 ± 1.34$^2$ (7.67)</td>
<td>8.17 ± 1.05 (8.09)</td>
<td>0.97</td>
<td>1.02</td>
<td></td>
</tr>
<tr>
<td>$CC$ ($n=21$)</td>
<td>8.36 ± 1.68 (8.21)</td>
<td>8.35 ± 1.90 (8.16)</td>
<td>8.59 ± 1.88 (8.41)</td>
<td>0.99</td>
<td>1.02</td>
<td></td>
</tr>
</tbody>
</table>

$^1$ ± SD; geometric mean in parentheses. FA group: 400 μg FA/d; MTHF group: 480 μg MTHF/d.

$^2$ The geometric mean at week 4 or 8 divided by the geometric mean at week 0 (baseline); values < 1 indicate a decrease, values = 1 indicate no change, and values > 1 indicate an increase after vitamin treatment.

$^3$ Significantly different from week 0 (paired t test with a Bonferroni-corrected $P$ value of 0.025): $^4 P < 0.001$, $^4 P < 0.025$.

$^5$ Data from one participant missing.
Supplemented with a microbiological assay in a subgroup of 20 women with the CT genotype (10 in the FA group and 10 in the MTHF group). The data shown in Table 5 are only reported descriptively. With the HPLC method, unmetabolized FA was detected after 8 wk in 8 of the 10 women in the FA group at ~1% of the total plasma folate concentration, but unmetabolized FA was not detected in the MTHF group. Other metabolites, such as tetrahydrofolate and 5-formyltetrahydrofolate, were detected in both the FA and MTHF groups but could not be quantified because their concentrations were below the detection limit of the method. RBC folate was measured before (week 0) and after treatment (week 8). Two-way ANOVA indicated a significant influence on RBC folate concentration of treatment group and of genotype (week 8). Two-way ANOVA indicated a significant influence on RBC folate concentration of treatment group and of genotype (week 8). With the HPLC method, unmetabolized FA was detected with a microbiological assay in a subgroup of 20 women with the CT genotype (10 in the FA group and 10 in the MTHF group). The data shown in Table 5 are only reported descriptively. With the HPLC method, unmetabolized FA was detected after 8 wk in 8 of the 10 women in the FA group at ~1% of the total plasma folate concentration, but unmetabolized FA was not detected in the MTHF group. Other metabolites, such as tetrahydrofolate and 5-formyltetrahydrofolate, were detected in both the FA and MTHF groups but could not be quantified because their concentrations were below the detection limit of the method.

<table>
<thead>
<tr>
<th>Table 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution of folate metabolites in plasma and plasma folate response to supplementation with folic acid (FA) or 5-methyltetrahydrofolate (MTHF) in women with the CT genotype.</td>
</tr>
<tr>
<td>Week 0</td>
</tr>
<tr>
<td>n mole/L</td>
</tr>
<tr>
<td>FA group (n = 51)</td>
</tr>
<tr>
<td>Total plasma folate</td>
</tr>
<tr>
<td>Total plasma folate</td>
</tr>
<tr>
<td>5-Methyltetrahydrofolate</td>
</tr>
<tr>
<td>Unmetabolized folic acid</td>
</tr>
<tr>
<td>Tetrahydrofolate</td>
</tr>
<tr>
<td>5-Formyltetrahydrofolate</td>
</tr>
<tr>
<td>MTHF group (n = 51)</td>
</tr>
<tr>
<td>Total plasma folate</td>
</tr>
<tr>
<td>Total plasma folate</td>
</tr>
<tr>
<td>5-Methyltetrahydrofolate</td>
</tr>
<tr>
<td>Unmetabolized folic acid</td>
</tr>
<tr>
<td>Tetrahydrofolate</td>
</tr>
<tr>
<td>5-Formyltetrahydrofolate</td>
</tr>
</tbody>
</table>

1 Data measured with the use of the immunoassay kit for the IMx analyzer (Abbott, Wiesbaden, Germany). 
2 Data measured with the use of HPLC coupled with a microbiological assay. 
3 Detected but not quantified.
in which women with the TT genotype had the greatest decrease (41%) in median fasting tHcy concentrations after 8 wk of supplementation with 500 μg FA/d. This result of greatest tHcy decrease in subjects with the TT genotype was also observed after supplementation with MTHF in our study, in which tHcy concentrations were reduced by 15% and 12% after 4 and 8 wk, respectively. Most likely because of the small group size, this observed effect did not reach statistical significance and should therefore be considered with caution.

Regarding the effects of vitamin application for each treatment group in total, we conclude that supplementation with 400 μg FA/d significantly reduces mean plasma tHcy concentrations (12% and 13% after 4 and 8 wk, respectively). Other researchers reported similar results in young women after 4 wk (11% reduction with 400 μg FA/d) (23, 24). The extent of reduction is known to be correlated with pretreatment tHcy concentrations and is more pronounced in subjects with high initial tHcy concentrations (23). Thus, the finding that the reduction in tHcy after 8 wk of FA supplementation was lower in our normohomocysteinemic subjects does not contrast with the findings of others (42, 43). Supplementation with the equimolar amount of 480 μg MTHF/d significantly reduced the mean plasma tHcy concentration by 7% after 4 wk, whereas no significant change (decrease of 3%) was observed after 8 wk. One possible explanation for the lower relative reduction after MTHF supplementation than after FA supplementation may be the use of the racemic mixture of MTHF. A priori, any effect of the unphysiologic 6R isomer cannot be excluded. However, assuming biological activity only for the 6S isomer (44) would imply that only one-half of the amount of MTHF administered in our study would have been active.

To our knowledge, this is the first study to report the effect of low-dose MTHF supplementation on plasma tHcy concentrations in healthy subjects. Bostom et al (30) recently reported a mean relative reduction of 17% in predialysis tHcy concentrations in 25 hemodialysis patients who were administered 17 mg L-5-MTHF/d orally for 12 wk. ŠD’Angelo et al (31) observed a 61% reduction in tHcy concentrations in 24 thrombophilic patients with hyperhomocysteinemia and the TT genotype who received oral supplementation with 15 mg racemic MTHF/d over 4 wk. In a study that did not include control subjects, Perna et al (32) found a mean reduction of 72% in predialysis tHcy concentrations in 14 uremic hemodialysis patients after they received oral supplementation with 15 mg racemic MTHF/d over 8 wk.

In the present study, the 3 genotype groups did not differ significantly in tHcy concentration before supplementation, although other researchers (17) showed that subjects with the TT genotype have, on average, 25% higher plasma tHcy concentrations than do subjects with the CC genotype. However, in previous studies an association of the TT genotype with elevated plasma tHcy concentrations was observed only when folate status was suboptimal (11, 12, 45), eg, when folate concentrations fell within the lowest quartile of plasma folate concentrations (17). Thus, we conclude that the absence of elevations in tHcy concentrations in the women with the TT genotype in our study was due to their adequate folate status. Although the women with the TT genotype had significantly lower plasma folate concentrations than did the women with the CT or CC genotype, their mean plasma folate concentration was above the lowest quintile in the range of values for the whole group at baseline. Additionally, plasma folate is not the preferred variable for the assessment of folate status. As a dynamic measure reflecting recent nutritional uptake, the plasma folate concentration fluctuates because of diet; thus, plasma folate is normally used as a marker for short-term folate status (46). RBC folate is regarded as a marker for long-term folate status because it reflects the folate status during erythropoiesis (46). In our study no significant differences in RBC folate concentrations between the genotype groups were seen at baseline. Concerning the gene-nutrient interaction in folate status, our study showed no influence of the genotype on an increase in plasma folate after supplementation with either folate derivative, but subjects with the TT genotype had an unexpected, greater increase in RBC folate than did subjects with the CT or CC genotypes.

In addition to the immunoassay method, HPLC coupled with a microbiological assay was used to identify the different folate metabolites in plasma samples. Because of the laborious procedure of plasma folate analysis by HPLC, only some of the samples were analyzed with this method. Unmetabolized FA appeared in plasma after oral administration of FA. The potential risk of masking pernicious anemia by metabolically unaltered FA is discussed in detail by Kelly et al (29). Because unmetabolized FA did not appear after MTHF supplementation, using MTHF in supplements and fortified foods may be a way to overcome this discussed, but unproven problem. However, the masking of the hematologic signs of vitamin B-12 deficiency through FA supplementation remains hypothetical and needs to be proven in future studies. Differences in plasma folate concentrations because of the measurement method used are sufficiently known and were intensively investigated (47, 48), but the marked deviations between the immunoassay and HPLC methods in plasma folate concentration after supplementation with MTHF were unexpected. Methodologic problems of the immunoassay with the racemic mixture of MTHF might be the underlying reason. The 6R isomer does not naturally occur in human plasma. Mader et al (44) reported an enhanced binding of this unphysiologic isomer to plasma proteins after intravenous infusion of racemic MTHF in cancer patients. We speculate that the unphysiologic 6R isomer was bound in the immunoassay and thus appeared active and measurable. This problem should be taken into account in future studies with racemic MTHF.

In summary, this randomized, double-blind, placebo-controlled study showed that the reduction in plasma tHcy concentration by FA or MTHF supplementation is influenced by a person’s MTHFR genotype. Subjects with the TT genotype may be able to compensate for the effects of the 677C→T mutation on homocysteine and folate metabolism if their folate status is adequate, eg, after intake of FA or MTHF.

We are grateful to the women who participated in the study. We thank P von Bülow, M Hages, G Puzicha, M Schüller, K Sielaff, and O Tobolski for excellent technical assistance and valuable discussions.

REFERENCES


27. CDC (Centers for Disease Control and Prevention). Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. Morb Mortal Wkly Rep 1992;41:2–8.


34. Brönnstrup A. Effects of single and combined B-vitamin supplementation on homocysteine concentrations in different population groups. PhD dissertation. Faculty of Agriculture, University of Bonn, Germany. Aachen, Germany: Wissenschaftlicher Fachverlag Dr. Fleck, 1995.


