

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

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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-methylenetetrahydrofolate 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 spon-

taneous 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-methylenetetrahydrofolate 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

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

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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 or foods enriched with vitamins.

The calculated size of the study population was based on our previous intervention studies in young women (34, 35), on the expected response of plasma tHcy to supplementation with 400 μ g FA/d, and on an anticipated dropout rate of 10%. Only the presumed effect of FA was taken into account because no data were available on the effect of a low-dose administration of MTHF on plasma tHcy. During the study 3 participants withdrew because of change of residence or personal reasons, and 3 participants were excluded 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. Fasting 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 μ g FA/d), MTHF (480 μ g 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 (calcium salt, racemic

mixture containing 50% each of the 6*S* and 6*R* 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 $2000 \times g$ 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 hemolysate 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 QIAamp DNA blood mini kit (Qiagen, Hilden, Germany); the polymerase chain reaction was performed according to the method of Frosst et al (14). All other

TABLE 1
Demographic characteristics of the study population¹

Characteristic	FA group (n = 51)	MTHF group (n = 52)	Placebo group (n = 57)	Total group (n = 160)
Age (y)	23.8 ± 3.5 ²	23.9 ± 3.9	23.9 ± 3.1	23.8 ± 3.5
BMI (kg/m ²)	20.7 ± 1.5	20.8 ± 1.7	21.3 ± 2.3	20.9 ± 1.9
Use of oral contraceptives (%)	68.6	59.6	64.9	64.4
Smoking (%)	19.6	15.4	21.1	18.8
Serum creatinine (μmol/L)	80.8 ± 12.3	82.3 ± 17.5	80.0 ± 13.9	81.0 ± 14.7
Plasma tHcy (μmol/L)	8.50 ± 2.71	8.88 ± 2.75	8.15 ± 1.66	8.50 ± 2.41
Plasma folate (nmol/L)	15.0 ± 4.9	16.5 ± 7.5	15.5 ± 7.0	15.7 ± 6.6
Red blood cell folate (nmol/L)	372 ± 132	422 ± 173	392 ± 135	395 ± 148
PLP (nmol/L)	51.1 ± 23.0	55.6 ± 41.0	47.4 ± 22.9	51.3 ± 30.0
Plasma vitamin B-12 (pmol/L)	232 ± 100	242 ± 131	237 ± 100	237 ± 110

¹FA, folic acid; MTHF, 5-methyltetrahydrofolate; tHcy, total homocysteine; PLP, pyridoxal-*P*. FA group: 400 μg FA/d; MTHF group: 480 μg MTHF/d. There were no significant differences between the 3 groups.

² $\bar{x} \pm SD$.

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. One-way ANOVA was used to compare the baseline concentrations of tHcy and plasma and RBC folate in the 3 genotype groups.

The primary analysis variable was the change in a plasma index after 4 and 8 wk of treatment. This change was expressed as the ratio of the concentrations at weeks 4 and 8, respectively, to that at week 0 (baseline). The ln-transformed ratio was tested by using Student's *t* test for within-subject comparisons and two-way ANOVA for between-subject comparisons. The factorial approach allows the main effects of treatment group and genotype to be tested and the possible interactions between these 2 factors to be investigated. In the case of significant interactions, further one-way ANOVAs were carried out and were stratified for treatment group or genotype to test differences between the genotypes or the 3 treatment groups, respectively. Post hoc

tests used Tukey's honestly significant difference procedure. Differences were considered significant at two-tailed *P* values < 0.05 except when analyzing both the 4- and 8-wk results by using Student's *t* test. In this case a Bonferroni-corrected *P* value of 0.025 (0.05/2) was applied to reduce the possibility of type I error. The software SPSS for WINDOWS (version 9.0; SPSS Inc, Chicago) was used for statistical analysis.

RESULTS

The demographic characteristics of the study population and the variables measured at baseline, including those for screening purposes, are shown in **Table 1**. The 3 treatment groups did not differ significantly in age, body mass index, 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 with genotype *CT* 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
Plasma total homocysteine (tHcy) and plasma and red blood cell folate concentrations at baseline stratified by *MTHFR* genotype¹

	<i>TT</i> (n = 21)	<i>CT</i> (n = 75)	<i>CC</i> (n = 64)
Plasma tHcy (μmol/L)	8.47 ± 3.52 (7.96)	8.50 ± 2.48 (8.21)	8.51 ± 1.86 (8.32)
Plasma folate (nmol/L)	12.1 ± 6.1 (10.9) ²	16.1 ± 6.7 (14.9)	16.4 ± 6.3 (15.5)
Red blood cell folate (nmol/L) ³	353 ± 162 (325)	405 ± 161 (378)	397 ± 125 (381)

¹ $\bar{x} \pm SD$; geometric mean in parentheses.

²Significantly different from the *CT* and *CC* genotypes, *P* < 0.001 (one-way ANOVA with Tukey's post hoc test).

³Data from 2 participants are missing (1 in the *TT* group and 1 in the *CC* group).

TABLE 3
Response of plasma total homocysteine to supplementation with folic acid (FA) and 5-methyltetrahydrofolate (MTHF) with respect to *MTHFR* genotype¹

	Week 0	Week 4	Week 8	Mean ratio ²	
				Week 4:0	Week 8:0
		<i>μmol/L</i>			
FA group (n = 51)	8.50 ± 2.71 (8.19)	7.43 ± 2.21 (7.17) ³	7.32 ± 1.73 (7.12) ³	0.88	0.87
TT (n = 5)	7.37 ± 1.44 (7.27)	5.89 ± 0.98 (5.83) ⁴	6.83 ± 0.92 (6.78)	0.80	0.93
CT (n = 25)	8.48 ± 3.23 (8.10)	7.09 ± 1.86 (6.91) ³	7.10 ± 1.67 (6.94) ⁴	0.85	0.86
CC (n = 21)	8.80 ± 2.24 (8.53)	8.21 ± 2.55 (7.87)	7.69 ± 1.92 (7.44) ⁴	0.92	0.87
MTHF group (n = 52)	8.88 ± 2.75 (8.52)	8.17 ± 1.85 (7.97) ⁴	8.51 ± 2.20 (8.25)	0.93	0.97
TT (n = 7)	10.40 ± 5.30 (9.40)	8.25 ± 2.25 (7.96)	8.71 ± 2.91 (8.30)	0.85	0.88
CT (n = 23)	8.91 ± 2.49 (8.57)	8.27 ± 2.13 (8.00) ⁴	8.91 ± 2.21 (8.67)	0.93	1.01
CC (n = 22)	8.37 ± 1.67 (8.22)	8.05 ± 1.43 (7.93)	8.02 ± 1.94 (7.83)	0.96	0.95
Placebo group (n = 57)	8.15 ± 1.66 (7.98)	8.09 ± 1.67 ⁵ (7.93)	8.51 ± 1.63 (8.36) ⁴	1.00	1.05
TT (n = 9)	7.58 ± 2.01 (7.36)	8.37 ± 1.98 (8.14)	8.92 ± 2.00 (8.70) ⁴	1.11	1.18
CT (n = 27)	8.06 ± 1.46 (7.93)	7.77 ± 1.34 ⁵ (7.67)	8.17 ± 1.05 (8.09)	0.97	1.02
CC (n = 21)	8.36 ± 1.68 (8.21)	8.35 ± 1.90 (8.16)	8.59 ± 1.88 (8.41)	0.99	1.02

¹ $\bar{x} \pm$ SD; geometric mean in parentheses. FA group: 400 μ g FA/d; MTHF group: 480 μ g MTHF/d.
²The 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.
^{3,4}Significantly different from week 0 (paired *t* test with a Bonferroni-corrected *P* value of 0.025): ³*P* < 0.001, ⁴*P* < 0.025.
⁵Data from one participant missing.

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 4

Response of plasma and red blood cell (RBC) folate to supplementation with folic acid (FA) and 5-methyltetrahydrofolate (MTHF)¹

	Week 0	Week 4	Week 8
FA group (n = 51)			
Plasma folate (nmol/L)	15.0 ± 4.9 (14.3)	24.3 ± 5.8 (23.5) ²	27.9 ± 8.0 (26.6) ²
RBC folate (nmol/L) ³	372 ± 132 (354)	—	608 ± 112 (598) ²
MTHF group (n = 52)			
Plasma folate (nmol/L)	16.5 ± 7.5 (15.0)	72.9 ± 22.8 (69.3) ²	82.3 ± 20.9 (79.8) ²
RBC folate (nmol/L) ^{3,4}	422 ± 173 (391)	—	674 ± 204 (647) ²
Placebo group (n = 57)			
Plasma folate (nmol/L)	15.5 ± 7.0 (14.3)	15.2 ± 5.2 ⁴ (14.4)	14.0 ± 4.6 (13.4)
RBC folate (nmol/L) ^{3,4}	392 ± 135 (373)	—	481 ± 133 (465) ²

¹ $\bar{x} \pm \text{SD}$; geometric mean in parentheses. FA group: 400 μg FA/d; MTHF group: 480 μg MTHF/d.²Significantly different from week 0, $P < 0.001$ (paired t test with a Bonferroni-corrected P value of 0.025).³Not measured in week 4.⁴Data from one participant missing.

pled 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 $\approx 1\%$ 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 ($P < 0.001$ for each), but the interaction between these 2 factors was not significant. The results are presented by treatment group only (Table 4). Geometric mean increases in RBC folate concentrations were significant and comparable in the FA and MTHF groups (69% and 66%, respectively). A significant increase was

observed in the placebo group also. The difference in the change in RBC concentration between each of the 2 vitamin groups and the placebo group was significant ($P < 0.001$; two-way ANOVA). Regarding genotype, the women with the *TT* genotype showed a more pronounced increase in mean RBC folate concentration (84%) than did the women with the *CT* genotype (50%) or the *CC* genotype (42%), and this difference was significant ($P < 0.001$; two-way ANOVA).

DISCUSSION

Plasma tHcy concentration is determined by genetic and nutritional factors. An inverse association between homocysteine concentration and folate status has been shown, and supplementation with folic acid is highly effective in lowering plasma tHcy concentrations (21). In the present study, we evaluated the efficacy of 2 folate derivatives, FA and MTHF, in lowering plasma tHcy concentrations in healthy female volunteers with respect to *MTHFR* polymorphism. To control for the effect of the 677C→T mutation in the *MTHFR* gene on any change in tHcy concentration, the study participants were randomly assigned according to genotype. The observed frequency of the various genotypes in our study population (13% *TT*, 47% *CT*, and 40% *CC*) agrees with the results of a meta-analysis (12% *TT*, 44% *CT*, and 44% *CC*) (17).

Our findings show that changes in tHcy concentrations depend on the form of folate used for supplementation and on the genotype. Supplementation with folic acid reduced plasma tHcy concentrations in all 3 genotype groups to a greater extent than did supplementation with MTHF. Our results further indicate that women with the *TT* genotype seem to benefit from supplementation with MTHF. One caveat must apply when referring to genotype. Because of randomization, the number of subjects with the *TT* genotype in each treatment group was quite small, and therefore the conclusions with respect to this genotype should be regarded as preliminary. After 4 wk of supplementation with 400 μg FA/d, we found decreases in tHcy concentrations of 8%, 15%, and 20% in subjects with the *CC*, *CT*, and *TT* genotypes, respectively. Malinow et al (16) observed similar reductions after supplementation with 1 and 2 mg FA/d for 3 wk: 7%, 13%, and 21% for subjects with the *CC*, *CT*, and *TT* genotypes, respectively. These data suggest that subjects with the *TT* genotype respond with a larger decrease in plasma tHcy concentration after FA supplementation than do individuals without the mutation. This was also shown in the study by Nelen et al (42),

TABLE 5

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* genotype¹

	Week 0	Week 8
	nmol/L	
FA group (n = 10)		
Total plasma folate ²	14.6 ± 6.1	25.7 ± 7.5
Total plasma folate ³	11.7 ± 7.7	29.3 ± 13.4
5-Methyltetrahydrofolate	11.7 ± 7.7	29.1 ± 13.5
Unmetabolized folic acid	0	0.2 ± 0.1
Tetrahydrofolate	— ⁴	—
5-Formyltetrahydrofolate	—	—
MTHF group (n = 10)		
Total plasma folate ²	15.8 ± 4.3	76.8 ± 17.2
Total plasma folate ³	12.1 ± 6.5	17.8 ± 6.4
5-Methyltetrahydrofolate	12.1 ± 6.5	17.8 ± 6.4
Unmetabolized folic acid	0	0
Tetrahydrofolate	—	—
5-Formyltetrahydrofolate	—	—

¹ $\bar{x} \pm \text{SD}$; FA group: 400 μg FA/d; MTHF group: 480 μg MTHF/d.²Measured with the use of the immunoassay kit for the IMx analyzer (Abbott, Wiesbaden, Germany).³Measured with the use of HPLC coupled with a microbiological assay.⁴Detected 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 μ g 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 μ g 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 μ g 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 μ g 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 6*R* isomer cannot be excluded. However, assuming biological activity only for the 6*S* 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. D'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 quartile 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 6*R* 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 6*R* 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.

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