



Supplementation with [6S]-5-methyltetrahydrofolate or folic acid equally reduces plasma total homocysteine concentrations in healthy women¹⁻³

Yvonne Lamers, Reinhold Prinz-Langenohl, Rudolf Moser, and Klaus Pietrzik

ABSTRACT

Background: Increased plasma total homocysteine (tHcy) is a risk factor for vascular disease and adverse pregnancy outcomes. Health authorities recommend periconceptional supplementation with 400 μg folic acid to prevent neural tube defects. Several countries have implemented food fortification with folic acid. However, excessive intake of folic acid could mask an undiagnosed vitamin B-12 deficiency. The biologically active [6S]-5-methyltetrahydrofolate ([6S]-5-MTHF) may be an alternative to folic acid because it is unlikely to mask vitamin B-12 deficiency symptoms.

Objective: We compared the tHcy-lowering potential of 2 dosages of [6S]-5-MTHF with that of 400 μg folic acid during 24 wk of supplementation.

Design: In this double-blind, randomized, controlled intervention trial, 144 female participants were supplemented daily with 400 μg folic acid, 416 μg [6S]-5-MTHF, 208 μg [6S]-5-MTHF, or placebo. Concentrations of tHcy and plasma folate were measured at baseline and at 4-wk intervals.

Results: After supplementation, there was a significant interaction between time and treatment with respect to changes in tHcy and plasma folate (both $P < 0.001$ by two-factor repeated-measures analysis of variance). The decrease in tHcy did not differ significantly between the 3 supplemented groups ($P > 0.05$; Tukey's post hoc test). The increase in plasma folate in the group receiving 208 μg [6S]-5-MTHF was significantly lower than that in the groups receiving 400 μg folic acid ($P < 0.001$) or 416 μg [6S]-5-MTHF ($P < 0.05$).

Conclusions: [6S]-5-MTHF was shown to be an adequate alternative to folic acid in reducing tHcy concentrations. Supplementation with 416 μg [6S]-5-MTHF was no more effective than that with 208 μg [6S]-5-MTHF. *Am J Clin Nutr* 2004;79:473-8.

KEY WORDS [6S]-5-methyltetrahydrofolate, folic acid, homocysteine, plasma folate, long-term effect, supplementation, healthy women

INTRODUCTION

Elevated plasma total homocysteine (tHcy) is a risk factor for vascular disease (1, 2), neurodegenerative disorders (3, 4), and adverse pregnancy outcomes such as preeclampsia, neural tube defects (NTDs), and placental abruption (5-7). High concentrations of tHcy are correlated with low blood folate, and increasing folate intake is a highly effective means of lowering tHcy. To reduce the occurrence of NTDs, public health authorities in sev-

eral countries recommend that women of childbearing age take $\geq 400 \mu\text{g}$ folic acid before and during the first trimester of pregnancy (8, 9). In view of a high number of unplanned pregnancies, some countries have implemented mandatory food fortification with folic acid (10, 11). Folic acid fortification has been associated with a decline in NTDs in the United States and Canada (12-14). In the United States, it has also been associated with a decrease in tHcy in the population (15), which may have an overall beneficial effect, eg, in terms of a reduction in vascular disease risk.

Folic acid fortification is not without risk: an excessive intake of folic acid may mask vitamin B-12 deficiency, potentially delaying its diagnosis (10). Folate and vitamin B-12 deficiency have the same hematologic symptom, megaloblastic anemia, which disappears after supplementation with large amounts of folic acid (10). However, the neurologic disorders associated with vitamin B-12 deficiency may progress unchecked (16, 17). Masking of vitamin B-12 deficiency is unique to folic acid. The predominant naturally occurring form of folate, [6S]-5-methyltetrahydrofolate ([6S]-5-MTHF), is unlikely to mask vitamin B-12 deficiency. The conversion of [6S]-5-MTHF into tetrahydrofolate, which is the precursor of folate forms involved in DNA synthesis, is vitamin B-12 dependent, whereas folic acid can be converted to tetrahydrofolate independent of vitamin B-12. Therefore, folic acid may maintain intracellular DNA synthesis and ameliorate megaloblastic anemia (16, 17). As a public health measure, [6S]-5-MTHF could possibly be used in food fortification or supplements as an alternative to folic acid, provided [6S]-5-MTHF has beneficial effects equal to those of folic acid with respect to reducing vascular disease risk and preventing NTDs.

¹ From the Institute of Nutritional Science, Department of Pathophysiology of Human Nutrition, University of Bonn, Germany (YL, RP-L, and KP), and Merck Eprova AG, Schaffhausen, Switzerland (RM).

² Supported by Merck KGaA (Darmstadt, Germany), which contributed financially, and by Merck Eprova AG (Schaffhausen, Switzerland), which provided Metafolin, the synthetic form of [6S]-5-methyltetrahydrofolate, for the supplements.

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

Received April 21, 2003.

Accepted for publication September 2, 2003.

On the basis of the recommendations for periconceptional supplementation with 400 μg folic acid to prevent NTDs (9), the aim of this double-blind, placebo-controlled trial was to compare the efficacy of a daily intake of 416 μg [6S]-5-MTHF with that of an equimolar amount of 400 μg folic acid in lowering tHcy in a group of healthy female subjects. To test whether lower amounts of [6S]-5-MTHF would be as effective as 400 μg folic acid, we assessed the dose response by adding to the study a group of subjects taking 208 μg [6S]-5-MTHF.

SUBJECTS AND METHODS

Subjects

Healthy women aged 18–35 y were recruited in December 2001 through advertisement at the University of Bonn, Germany. Inclusion criteria for participation in the study were normal results on routine laboratory tests and adequate vitamin B-12 status (plasma vitamin B-12 concentration ≥ 110 pmol/L; 18). Women were not eligible if they were pregnant, lactating, or planning a pregnancy in the next few months. Regular consumers of folic acid, defined as persons taking > 100 μg folic acid/d in vitamin supplements or fortified food during the last 4 mo, were not included. Further exclusion criteria were medical treatment interfering with folate metabolism (ie, cholestyramine, methotrexate, trimethoprim, sulfasalazine, salicylic acid, cotrimoxazole, antiacid, and antiepileptic drugs) and abuse of alcohol or drugs. The study was approved by the Ethics Committee of the Medical Association of Hamburg, Germany, and all participants gave written informed consent.

Design

The study was a 24-wk (January–July 2002), double-blind, randomized, placebo-controlled intervention trial with parallel group design. Because the 677C \rightarrow T polymorphism of the gene encoding for 5,10-methylenetetrahydrofolate reductase (*MTHFR*) has an effect on homocysteine metabolism with respect to the response to folate intake or folic acid supplementation (19, 20), participants were stratified according to their genotype before randomization to ensure equal distribution of the 3 genotype variants in all groups. Participants were randomly assigned to receive either a placebo, 400 μg folic acid, 416 μg [6S]-5-MTHF, or 208 μg [6S]-5-MTHF. Fasting blood samples were collected by venipuncture at baseline and at 4, 8, 12, 16, 20, and 24 wk of the study. Participants were asked to take one capsule every morning before breakfast, except on the blood-sampling days, when they were asked to take the capsule after venipuncture. Possible side effects were monitored by questioning the subjects at each visit and by reviewing routine laboratory variables. Compliance was assessed by counting the pills remaining at weeks 8, 16, and 24. Dietary intakes were assessed by using 3-d diet records completed at baseline and at weeks 8, 16, and 24 and analyzed by using EBISPRO for WINDOWS (version 4; J Erhardt, University of Hohenheim, Germany).

Supplements

The supplements were manufactured by PCI Services (Schorndorf, Germany) as hard gelatin capsules containing a blend of magnesium stearate and microcrystalline cellulose as a filler (placebo), 400 μg (906 nmol) folic acid (Caesar & Loretz GmbH, Hilden, Germany), or 416 μg (906 nmol) or 208 μg (453

nmol) [6S]-5-MTHF as calcium salt (Metafolin; Merck Eprova AG, Schaffhausen, Switzerland). Subjects and investigators were blinded to treatment. Stability tests of the supplements performed at the beginning, middle, and end of the intervention period showed a recovery of 98% of both folate forms.

Blood sampling and analysis

Blood samples for measurement of plasma folate, tHcy, and plasma vitamin B-12 concentrations were collected into EDTA-coated tubes (S-Monovette; Sarstedt, Nümbrecht, Germany), immediately put on ice, centrifuged within 15 min (3000 rpm for 10 min at 4 °C), and stored at -20 °C before analysis. Plasma folate and tHcy concentrations were measured by using an immunoassay on the Abbott AxSYM analyzer (Abbott, Wiesbaden, Germany). The intraassay and interassay CVs were 5.9% and 5.6% for plasma folate and 2.3% and 3.5% for tHcy, respectively, according to the results of the analyses of plasma pools and controls provided by the manufacturers. To avoid between-run variation, samples from each participant were measured in one run. Before the determination of plasma folate concentrations, blood samples were manually diluted (1:2) with the use of the AxSYM folate specimen diluent. Plasma vitamin B-12 was measured at baseline by using an immunoassay on the Abbott AxSYM analyzer. Routine laboratory variables including serum creatinine concentrations were measured after venipuncture, at baseline and at week 24, by the central laboratory of the University Hospital, Bonn. Serum creatinine concentrations were measured enzymatically by using the method of Jaffé (Dade Behring, Bad Schwallbach, Germany). Identification of the 677C \rightarrow T *MTHFR* genotype was conducted by using the polymerase chain reaction according to the method of Frosst et al (21).

Statistical methods

Before further analysis, data were checked for normal distribution with the use of the Shapiro-Wilk test. Because they were skewed positively, data were log transformed to normalize distribution for analyses and back-transformed to geometric means with 95% CI for presentation in tables. One-way analysis of variance (ANOVA) was used to compare the 4 groups with respect to baseline characteristics, dietary intake of folate and methionine, and compliance. Chi-square tests were used to evaluate categorical variables such as smoking and the use of oral contraceptives. For within-group comparison of dietary intake of folate and methionine at baseline and at week 24, Student's paired *t* test was used.

For further analyses, the natural logarithms of plasma folate and tHcy were used in all statistical tests as continuous variables. Two-factor repeated-measures ANOVA was applied to examine the interaction between time and treatment and to test for changes within time and treatment. Post hoc analysis was carried out with Tukey's honestly significant difference test. In case of significant time \times treatment interaction, the within-group comparison was carried out by using a Bonferroni corrected *P* value of 0.008 (0.05/6). Furthermore, multiple regression was used to determine mean percentage changes in plasma folate and tHcy after 24 wk relative to the placebo group after adjustment for baseline values (22). Between-group comparisons were carried out with the use of Tukey's post hoc test. Results were considered significant at *P* < 0.05. All analyses were undertaken by using SPSS for WINDOWS software (version 11; SPSS Inc, Chicago).

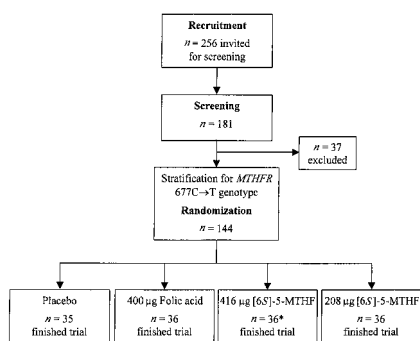


FIGURE 1. Flow chart of the study. *One participant withdrew after 4 wk of intervention. *MTHFR*, methylenetetrahydrofolate reductase; [6S]-5-MTHF, [6S]-5-methyltetrahydrofolate.

RESULTS

A flow chart of the study is presented in **Figure 1**. One hundred eighty-one women came to the screening blood test, 37 of whom were excluded because of regular consumption of folic acid ($n = 20$), plasma vitamin B-12 concentration < 110 pmol/L ($n = 9$), or personal reasons ($n = 8$). One hundred forty-four women were included in the study. During the intervention period, one participant withdrew for personal reasons. After the exclusion of one outlier (baseline tHcy: $> \times \pm 3$ SD) and 7 subjects with missing values (absence on blood-sampling days because of vacation or illness), data from 135 subjects were entered into statistical analyses.

Baseline characteristics of those 135 subjects are presented in **Table 1**. The 4 groups did not differ with respect to age, body mass index, use of oral contraceptives, or smoking. After randomization, all groups were homogeneous for the main factors, tHcy and plasma folate concentrations. Plasma vitamin B-12 concentrations did not differ significantly between the groups, and all participants had an adequate plasma vitamin B-12 status. Significant baseline differences were found only for serum creatinine, which is a marker of renal function; however, all subjects had serum creatinine concentrations within the normal range.

Dietary intakes of folate and methionine did not differ significantly between the groups at baseline and at week 24 or within the groups between these points in time ($P > 0.05$). At baseline, the mean (\pm SD) dietary intake of the study population was 257 ± 108 μ g dietary folate equivalents/d and 1.4 ± 0.5 g methionine/d. The supplements were well tolerated, and no side effects were reported. Compliance was high and did not differ significantly between the groups ($P > 0.05$). Ninety percent of the subjects consumed $\geq 95\%$ of the supplements, and the other 10% of subjects consumed 86–94% of the supplements.

The mean concentrations of tHcy and plasma folate at baseline and throughout the intervention period are shown in **Table 2**. For both tHcy and plasma folate, a significant interaction between time and treatment ($P < 0.001$) was determined by two-factor repeated-measures ANOVA. The reduction in tHcy during the intervention period did not differ significantly between the supplemented groups ($P > 0.05$; Tukey's post hoc test). Within-group comparisons found significant reductions in tHcy concentrations between baseline and week 4 and between week 4 and week 16 in the group taking 400 μ g folic acid. Subjects receiving 416 μ g or 208 μ g [6S]-5-MTHF had a significant decrease in tHcy concentration after 4 wk of intervention. After this period, tHcy concentrations in those two groups remained steady until week 24. Relative to tHcy in the placebo group, tHcy in the groups receiving folic acid or 416 μ g or 208 μ g [6S]-5-MTHF decreased significantly by 15% (95% CI: 8%, 21%), 19% (12%, 24%), and 19% (12%, 25%), respectively, after 24 wk.

Plasma folate concentrations increased significantly ($P < 0.001$) in all 3 supplemented groups, relative to the placebo group (Table 2). The increase in plasma folate in the group receiving 208 μ g [6S]-5-MTHF was significantly less than that in the groups receiving 400 μ g folic acid ($P < 0.001$) and 416 μ g [6S]-5-MTHF ($P < 0.05$). Within-group comparison found that plasma folate increased until week 8 in the group taking 400 μ g folic acid, and that it remained at that concentration until the end of the study. The group receiving 208 μ g [6S]-5-MTHF had an increase in plasma folate until week 12. Only in the group receiving 416 μ g [6S]-5-MTHF did plasma folate rise continu-

TABLE 1
Baseline characteristics of the study population¹

	Treatment group			
	Placebo (n = 34)	400 μ g Folic acid (n = 34)	416 μ g [6S]-5-MTHF (n = 35)	208 μ g [6S]-5-MTHF (n = 32)
Age (y)	22.6 \pm 2.4 ²	23.6 \pm 3.2	24.2 \pm 4.0	22.9 \pm 2.4
BMI (kg/m ²)	21.3 \pm 1.8	20.7 \pm 2.4	21.6 \pm 3.0	21.0 \pm 2.4
Plasma tHcy (μ mol/L)	8.1 (7.5, 8.7) ³	8.2 (7.6, 8.7)	8.3 (7.6, 9.0)	8.1 (7.4, 8.9)
Plasma folate (nmol/L)	14.1 (12.6, 15.7)	14.4 (12.6, 16.5)	13.3 (11.9, 14.9)	14.6 (12.9, 16.5)
Plasma vitamin B-12 (pmol/L)	225 (197, 255)	216 (195, 240)	239 (214, 267)	210 (191, 231)
Serum creatinine (μ mol/L)	74.6 (71.1, 78.1)	71.6 (68.9, 74.4)	74.7 (71.7, 77.9)	67.6 (64.4, 71.0) ⁴
<i>MTHFR</i> 677C→T (n)				
CC	16	15	16	14
CT	15	16	16	14
TT	3	3	3	4
Use of oral contraceptives (%)	65	65	63	78
Smoking (%)	24	21	29	22

¹ [6S]-5-MTHF, [6S]-5-methylenetetrahydrofolate; tHcy, total homocysteine; *MTHFR*, methylenetetrahydrofolate reductase.

² Arithmetic $\bar{x} \pm$ SD.

³ Geometric \bar{x} ; 95% CI in parentheses.

⁴ Significantly different from the placebo and 416 μ g [6S]-5-MTHF groups, $P < 0.05$ (one-way ANOVA with Bonferroni post hoc test).



TABLE 2
Plasma total homocysteine (tHcy) and plasma folate concentrations at baseline and over the entire intervention period¹

Substance and treatment group	Baseline	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Estimated change after 24 wk (%) ²
Plasma tHcy ($\mu\text{mol/L}$)								
Placebo ($n = 34$)	8.1 (7.5, 8.7)	8.4 (7.8, 9.0)	8.1 (7.6, 8.6)	8.4 (7.8, 9.0)	8.3 (7.7, 8.9)	8.3 (7.7, 9.0)	8.3 (7.6, 9.0)	—
400 μg Folic acid ($n = 34$)	8.2 (7.6, 8.7)	7.2 (6.8, 7.6) ³	6.8 (6.5, 7.3) ³	6.8 (6.4, 7.3) ³	6.7 (6.3, 7.1) ^{3,4}	6.9 (6.4, 7.3) ³	7.1 (6.6, 7.5) ³	-15 (-8, -21) ⁵
416 μg [6S]-5-MTHF ($n = 35$)	8.3 (7.6, 9.0)	6.7 (6.2, 7.2) ³	6.7 (6.2, 7.3) ³	6.5 (6.0, 7.0) ³	6.5 (6.1, 7.1) ³	6.6 (6.2, 7.1) ³	6.8 (6.4, 7.2) ³	-19 (-12, -24) ⁵
208 μg [6S]-5-MTHF ($n = 32$)	8.1 (7.4, 8.9)	7.1 (6.5, 7.6) ³	7.1 (6.6, 7.7) ³	7.0 (6.5, 7.5) ³	6.8 (6.3, 7.3) ³	6.8 (6.3, 7.4) ³	6.7 (6.2, 7.3) ³	-19 (-12, -25) ⁵
Plasma folate (nmol/L)								
Placebo ($n = 34$)	14.1 (12.6, 15.7)	14.2 (12.7, 16.0)	13.2 (11.6, 14.9)	13.2 (11.7, 15.0)	14.0 (12.2, 15.9)	14.5 (12.5, 17.0)	13.7 (11.9, 15.8)	—
400 μg Folic acid ($n = 34$)	14.4 (12.6, 16.5)	28.8 (26.3, 31.5) ³	34.9 (32.6, 37.4) ^{3,4}	35.7 (33.4, 38.0) ^{3,4}	37.1 (34.4, 40.0) ^{3,4}	37.6 (35.5, 39.9) ^{3,4}	34.7 (32.6, 37.0) ^{3,4}	151 (119, 188) ⁵
416 μg [6S]-5-MTHF ($n = 35$)	13.3 (11.9, 14.9)	24.5 (21.9, 27.4) ³	30.6 (27.5, 34.0) ^{3,4}	32.6 (29.4, 36.2) ^{3,4}	35.6 (32.6, 38.9) ^{3,4,6}	37.4 (34.4, 40.6) ^{3,4,6,7}	35.5 (32.2, 39.1) ^{3,4}	164 (130, 203) ⁵
208 μg [6S]-5-MTHF ($n = 32$)	14.6 (12.9, 16.5)	21.5 (19.0, 24.3) ³	24.4 (21.9, 27.1) ³	25.3 (22.2, 28.8) ^{3,4}	27.8 (25.2, 30.7) ^{3,4}	27.5 (24.6, 30.8) ^{3,4}	27.9 (24.8, 31.3) ^{3,4}	101 (75, 131) ^{5,8}

¹ Geometric \bar{x} ; 95% CI in parentheses. [6S]-5-MTHF, [6S]-5-methyltetrahydrofolate. There were significant time \times treatment interactions for changes in plasma tHcy and plasma folate (both $P < 0.001$, two-factor repeated-measures ANOVA). There were significant differences between the placebo group and all 3 treatment groups in changes in plasma tHcy ($P < 0.01$) and plasma folate ($P < 0.001$) (Tukey's post hoc test) and between the group receiving 208 μg [6S]-5-MTHF and the groups receiving 400 μg folic acid ($P < 0.001$) and 416 μg [6S]-5-MTHF ($P < 0.05$) in changes in plasma folate (Tukey's post hoc test).

² Relative to the placebo group after adjustment for baseline concentration.

³ Significantly different from baseline after Bonferroni correction, $P < 0.008$.

⁴ Significantly different from week 4 after Bonferroni correction, $P < 0.008$.

⁵ Significantly different from the placebo group after adjustment for baseline concentration, $P < 0.01$ (Tukey's post hoc test).

⁶ Significantly different from week 8 after Bonferroni correction, $P < 0.008$.

⁷ Significantly different from week 12 after Bonferroni correction, $P < 0.008$.

⁸ Significantly different from the 416 μg [6S]-5-MTHF group after adjustment for baseline concentration, $P < 0.01$ (Tukey's post hoc test).

ously until week 20. Multiple regression showed a significant mean percentage increase in plasma folate in all 3 supplemented groups relative to the placebo group. Plasma folate increased by 151% (95% CI: 119%, 188%), 164% (95% CI: 130%, 203%), and 101% (95% CI: 75%, 131%) in the groups receiving 400 μ g folic acid and 416 μ g and 208 μ g [6S]-5-MTHF, respectively, after 24 wk.

DISCUSSION

This study is the first to investigate the long-term effect of different amounts of [6S]-5-MTHF on tHcy in healthy females. Relative to the placebo group, tHcy decreased by 15%, 19%, and 19% in the groups receiving 400 μ g folic acid or 416 μ g or 208 μ g [6S]-5-MTHF, respectively, after 24 wk. The tHcy reduction did not differ significantly between the groups receiving supplementation with 400 μ g folic acid and the equimolar amount of [6S]-5-MTHF. As expected in a group of healthy young women, tHcy concentration decreased by $\leq 20\%$ (23, 24) after supplementation with 400 μ g folic acid. Intervention with 416 μ g [6S]-5-MTHF was no more effective than was that with 208 μ g [6S]-5-MTHF in lowering the tHcy concentration. Therefore, a 208 μ g dose of [6S]-5-MTHF seems sufficient to reduce tHcy concentrations in a group of healthy female subjects.

The effect of [6S]-5-MTHF supplementation on lowering tHcy was not investigated until recently. Venn et al (25) reported the effect of 113 μ g [6S]-5-MTHF on tHcy concentrations. Twenty-four weeks of that intervention led to a 15% reduction in tHcy, which was significantly greater than the 9% reduction in the group receiving 100 μ g folic acid (25). Throughout the study period of 24 wk, tHcy concentrations decreased continuously in both supplemented groups; it therefore remains unknown whether the maximum reduction in tHcy was achieved by supplementation with 113 μ g [6S]-5-MTHF after 24 wk. In the present study, the decrease in tHcy concentrations reached a maximum after 4 wk ($P < 0.001$), and there were no further reductions until 24 wk, irrespective of the form and dose of folate. Baseline tHcy concentrations in both study populations were similar [8.6 μ mol/L (25) and 8.2 μ mol/L, respectively], which allowed us to compare the reductions in tHcy concentration resulting from supplementation with 113, 208, and 416 μ g [6S]-5-MTHF after 24 wk. These reductions were 15% (25), 19%, and 19%, respectively, which indicated that there was no significant difference in tHcy reduction between the 3 interventions with [6S]-5-MTHF. However, further trials are needed to compare the efficacy of 113, 208, and 416 μ g [6S]-5-MTHF in one study population.


Supplementation with folate derivatives improves folate concentration in plasma. In the present study, plasma folate concentrations increased significantly by 151%, 164%, and 101% in the groups receiving 400 μ g folic acid or 416 μ g or 208 μ g [6S]-5-MTHF, respectively, after 24 wk, relative to the control group. Supplementation with 416 μ g [6S]-5-MTHF and an equimolar amount of folic acid affected plasma folate equally. This finding is in accordance with the results of Venn et al (26). In the present study, a steady state was defined as no significant increase in plasma folate beyond that reached after 24 wk of intervention. However, that same concentration was reached after only 8 wk of supplementation with 400 μ g folic acid. Thus, plasma folate was stabilized at an earlier stage by folic acid than by supplementation with 208 μ g and 416 μ g [6S]-5-MTHF, with which a steady

state in plasma folate concentrations was reached after 12 and 20 wk, respectively. In the study by Venn et al (26), no steady state plateau was reached after supplementation with 113 μ g [6S]-5-MTHF and 100 μ g folic acid over 24 wk. However, the amount of folate derivatives given may have been too low, or the observation period may have been too short.

Plasma folate is a strong determinant of tHcy concentration (27). Although supplementation with 208 μ g [6S]-5-MTHF resulted in a different plasma folate concentration than did that with 416 μ g [6S]-5-MTHF, both decreased tHcy equally. Assuming [6S]-5-MTHF to be the active form of folate in the metabolism (28) and the main form occurring naturally in food (29, 30), the finding of an equal tHcy-lowering potential of 400 μ g folic acid and 208 μ g [6S]-5-MTHF, despite a lesser increase in plasma folate after supplementation with the latter is comparable to the finding of the study by Ashfield-Watt et al (19). In their investigation, a diet high in natural folate did not increase plasma folate to the same extent as did supplementation with 400 μ g folic acid over a period of 4 mo. However, the decrease in tHcy observed in the group consuming the folate-rich diet equalled that in the group receiving folic acid supplements (19). Boushey et al (2) reported that a lower plateau of tHcy is reached when plasma folate concentrations exceed 15 nmol/L. These findings match results of the present study, as plasma folate between baseline and week 4 increased to > 15 nmol/L in all 3 supplemented groups, whereas tHcy concentrations decreased concurrently. However, the study of Venn et al (25) found a reduction in tHcy even though plasma folate concentrations at baseline were already > 15 nmol/L. In the present study, plasma folate concentrations continued increasing in all 3 supplemented groups after 4 wk, and tHcy stabilized at lower concentrations after 4 wk in this supplemented group of healthy women. The effect of plasma folate concentrations on tHcy seems to diminish at plasma folate concentrations that signify a sufficient folate supply.

[6S]-5-MTHF may be an adequate alternative to folic acid because it is unlikely to mask the hematologic symptoms of vitamin B-12 deficiency. In view of the increasing numbers of elderly in the population and the high prevalence of vitamin B-12 deficiency in that group (31), the possible masking of the symptoms by high folic acid intakes is exceptionally relevant. Where mandatory food fortification with folic acid has been implemented, some people reach a daily intake of folic acid > 1 mg (32), which is the tolerable upper intake level above which masking of vitamin B-12 deficiency has been determined to occur (10). [6S]-5-MTHF could be an alternative derivative for supplementation or fortification. In the supplemental form offered as calcium salt in the present study, [6S]-5-MTHF was stable throughout the study period. Good stability of [6S]-5-MTHF in some processed foods (cereals, white bread) was reported by the manufacturer. However, further tests of stability should be performed for different kinds of processed foods (26). The safety of [6S]-5-MTHF was shown in the study by Bostom et al (33) that investigated daily supplementation with 17 mg [6S]-5-MTHF over 12 wk with respect to reducing tHcy in hemodialysis patients. No side effects were reported. With respect to food fortification with [6S]-5-MTHF, intervention trials are needed to evaluate the minimum effective dose of supplemented [6S]-5-MTHF out of food to lower tHcy concentrations.

In conclusion, the present study showed that supplementation with both 208 and 416 μ g [6S]-5-MTHF significantly decreased

tHcy and significantly increased plasma folate concentrations. [6S]-5-MTHF did not differ in tHcy-lowering potential from an equimolar amount of folic acid. Supplementation with 416 μg [6S]-5-MTHF was no more effective in lowering tHcy concentrations than was that with 208 μg [6S]-5-MTHF. In addition to the observed beneficial effect on tHcy and plasma folate, [6S]-5-MTHF is unlikely to mask vitamin B-12 deficiency as folic acid does. Because this folate form is also stable and is as bioavailable as folic acid, it may be an adequate alternative to folic acid for use in food fortification or supplements. Further studies should be undertaken to investigate the effect of [6S]-5-MTHF in women and men of other age groups and especially in those with elevated tHcy concentrations. Such trials should include dose-response assessments as were used in the present study. 

We acknowledge the women who participated in the study. We thank S Braemswig, A Brönstrup, P von Bülow, S Deneke, I Fohr, M Hages, M Schüller, P Pickert, G Puzicha, and O Tobolski for excellent technical assistance and valuable discussions.

YL and KP had the original idea for the study. All authors were responsible for designing and planning the study. YL and KP recruited subjects. YL was responsible for sample collection and laboratory and statistical analyses. All authors contributed to writing the manuscript. RM is the Chief Scientific Officer for Merck Eprova AG, Switzerland, the company that manufactures Metafolin, which it provided for this study. RM contributed to the study by participating in planning the project and writing the manuscript. The stability tests for the supplements were performed by Merck Eprova AG under RM's supervision. RM was not involved in any decisions about laboratory work and statistical analysis or publication. YL, RP-L, and KP declared no conflict of interest.

REFERENCES

- Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *BMJ* 2002;325:1202–06.
- Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *JAMA* 1995;274:1049–57.
- Mattson MP, Kruman II, Duan W. Folic acid and homocysteine in age-related disease. *Ageing Res Rev* 2002;1:95–111.
- Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med* 2002;346:476–83.
- Vollset SE, Refsum H, Irgens LM, et al. Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine Study. *Am J Clin Nutr* 2000;71:962–8.
- Ray JG, Laskin CA. Folic acid and homocyst(e)ine metabolic defects and the risk of placental abruption, pre-eclampsia and spontaneous pregnancy loss: a systematic review. *Placenta* 1999;20:519–29.
- Mills JL, McPartlin JM, Kirke PN, et al. Homocysteine metabolism in pregnancies complicated by neural-tube defects. *Lancet* 1995;345:149–51.
- Cornel MC, Erickson JD. Comparison of national policies on periconceptional use of folic acid to prevent spina bifida and anencephaly (SBA). *Teratology* 1997;55:134–7.
- Commission of the European Communities. Nutrient and energy intakes for the European Community. Reports of the Scientific Committee for Food. 31st series. Luxembourg: Office for Official Publications of the European Communities, 1993.
- Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, other B Vitamins, and Choline and Subcommittee on Upper Reference Levels of Nutrients, Food and Nutrition Board, Institute of Medicine. Folate. In: *Dietary intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin and choline*. Washington, DC: National Academy Press, 1998:196–305.
- Health Canada. Food and Drug Regulations, Amendment Schedule No. 1066. Ottawa: Health Canada, 1997.
- Honein MA, Paulozzi LJ, Mathews TJ, Erickson JD, Wong LY. Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. *JAMA* 2001;285:2981–6.
- Persad VL, Van den Hof MC, Dube JM, Zimmer P. Incidence of open neural tube defects in Nova Scotia after folic acid fortification. *Can Med Assoc J* 2002;167:241–5.
- Ray JG, Meier C, Vermeulen MJ, Boss S, Wyatt PR, Cole DE. Association of neural tube defects and folic acid food fortification in Canada. *Lancet* 2002;360:2047–8.
- Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *N Engl J Med* 1999;340:1449–54.
- Weir DG, Scott JM. Brain function in the elderly: role of vitamin B12 and folate. *Br Med Bull* 1999;55:669–82.
- Scott JM, Weir DG. The methyl folate trap. A physiological response in man to prevent methyl group deficiency in kwashiorkor (methionine deficiency) and an explanation for folic-acid induced exacerbation of subacute combined degeneration in pernicious anaemia. *Lancet* 1981;2:337–40.
- Herbert V, Das KC. Folic acid and vitamin B12. In: Shils ME, Olson JA, Shike M, eds. *Modern nutrition in health and disease*. Philadelphia: Lea & Febiger, 1994:402–25.
- Ashfield-Watt PA, Pullin CH, Whiting JM, et al. Methylene tetrahydrofolate reductase 677C→T genotype modulates homocysteine responses to a folate-rich diet or a low-dose folic acid supplement: a randomized controlled trial. *Am J Clin Nutr* 2002;76:180–6.
- Bailey LB, Gregory JF III. Polymorphisms of methylenetetrahydrofolate reductase and other enzymes: metabolic significance, risks and impact on folate requirement. *J Nutr* 1999;129:919–22.
- Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995;10:111–3.
- Vickers AJ, Altman DG. Statistics notes: analysing controlled trials with baseline and follow up measurements. *BMJ* 2001;323:1123–4.
- Fohr IP, Prinz-Langenohl R, Brönstrup A, et al. 5,10-Methylenetetrahydrofolate reductase genotype determines the plasma homocysteine-lowering effect of supplementation with 5-methyltetrahydrofolate or folic acid in healthy young women. *Am J Clin Nutr* 2002;75:275–82.
- Brönstrup A, Hages M, Prinz-Langenohl R, Pietrzik K. Effects of folic acid and combinations of folic acid and vitamin B-12 on plasma homocysteine concentrations in healthy, young women. *Am J Clin Nutr* 1998;68:1104–10.
- Venn BJ, Green TJ, Moser R, Mann JI. Comparison of the effect of low-dose supplementation with L-5-methyltetrahydrofolate or folic acid on plasma homocysteine: a randomized placebo-controlled study. *Am J Clin Nutr* 2003;77:658–62.
- Venn BJ, Green TJ, Moser R, McKenzie JE, Skeaff CM, Mann J. Increases in blood folate indices are similar in women of childbearing age supplemented with [6S]-5-methyltetrahydrofolate and folic acid. *J Nutr* 2002;132:3353–5.
- Jacques PF, Bostom AG, Wilson PW, Rich S, Rosenberg IH, Selhub J. Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. *Am J Clin Nutr* 2001;73:613–21.
- Mader RM, Steger GG, Rizovski B, et al. Stereospecific pharmacokinetics of rac-5-methyltetrahydrofolic acid in patients with advanced colorectal cancer. *Br J Clin Pharmacol* 1995;40:209–15.
- Muller H. [Determination of the folic acid content of vegetables and fruits using high-performance liquid chromatography (HPLC)]. *Z Lebensmittelchem* 1993;196:137–41 (in German).
- Stralsjo L, Arkbage K, Witthoft CM, Jagerstad M. Evaluation of a radioprotein-binding assay (RPBA) for folate analysis in berries and milk. *Food Chemistry* 2002;79:525–34.
- Baik HW, Russell RM. Vitamin B12 deficiency in the elderly. *Annu Rev Nutr* 1999;19:357–77.
- Lewis CJ, Crane NT, Wilson DB, Yetley EA. Estimated folate intakes: data updated to reflect food fortification, increased bioavailability, and dietary supplement use. *Am J Clin Nutr* 1999;70:198–207.
- Bostom AG, Shemin D, Bagley P, et al. Controlled comparison of L-5-methyltetrahydrofolate versus folic acid for the treatment of hyperhomocysteinemia in hemodialysis patients. *Circulation* 2000;101:2829–32.