

Comparison of the effect of low-dose supplementation with L-5-methyltetrahydrofolate or folic acid on plasma homocysteine: a randomized placebo-controlled study¹⁻³

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ABSTRACT

Background: Food fortification with folic acid has been introduced in several countries for the prevention of neural tube defects. Fortification has lowered total homocysteine (tHcy) concentrations in the US population, a consequence that may have health benefits. However, folic acid fortification could mask vitamin B-12 deficiency. Synthetic L-5-methyltetrahydrofolate (L-MTHF) may be more appropriate than folic acid as a fortificant because it is unlikely to mask the hematologic indicators of vitamin B-12 deficiency.

Objective: The objective of the study was to compare the effectiveness of 100 µg folic acid/d with that of equimolar L-MTHF in lowering tHcy in healthy volunteers.

Design: The study was designed as a 24-wk, randomized, placebo-controlled intervention. Free-living healthy volunteers ($n = 167$) were randomly assigned to receive a daily supplement containing folic acid (100 µg), L-MTHF (113 µg), or placebo. Blood collected at baseline and at 8, 16, and 24 wk was analyzed for tHcy, plasma folate, and red blood cell folate (RCF) concentrations.

Results: At 24 wk, after adjustment for baseline values, mean (95% CI) tHcy was 14.6% (9.3, 19.5%) and 9.3% (3.7, 14.6%) lower, mean plasma folate was 34% (14, 56%) and 52% (30, 78%) higher, and mean RCF was 23% (12, 35%) and 31% (19, 44%) higher in the L-MTHF and folic acid groups, respectively, than in the placebo group. L-MTHF was more effective than was folic acid in lowering tHcy ($P < 0.05$). At 24 wk, the increases in plasma folate and RCF concentrations did not differ significantly between the 2 supplemented groups.

Conclusion: Low-dose L-MTHF is at least as effective as is folic acid in reducing tHcy concentrations in healthy persons. *Am J Clin Nutr* 2003;77:658–62.

KEY WORDS L-5-Methyltetrahydrofolate, L-MTHF, folic acid, homocysteine, plasma folate, red blood cell folate, cardiovascular disease, clinical trial

INTRODUCTION

Health authorities in several countries recommend that women planning a pregnancy take a supplement of ≥ 400 µg folic acid/d to reduce the risk of having an infant with a neural tube defect (NTD) (1). Because many pregnancies are unplanned and the neural tube closes early in pregnancy (ie, before women may know they are pregnant), mandatory food fortification with folic acid has been introduced in some countries (2, 3) and is being con-

sidered in others (4, 5). Data from the United States indicate a 19% reduction in NTD prevalence after the implementation of mandatory folic acid fortification in 1998 (6). A further benefit of increasing folate consumption may be its effect on plasma total homocysteine (tHcy), an amino acid associated with an increased risk of occlusive vascular disease (7). Indeed, folate has been shown to improve vascular endothelial function in patients with coronary artery disease, and folate in combination with other B vitamins decreased restenosis after coronary angioplasty (8, 9). Folic acid fortification in the United States has been shown to lower tHcy concentration, which may have a public health benefit (10).

Folic acid, a synthetic oxidized form of folate, is used in supplements and added to food because of its high stability and bioavailability. A major concern related to excessive intakes of folic acid is that of possible masking of hematologic signs of vitamin B-12 deficiency, which might delay diagnosis and thus allow the progression of neurologic damage (11, 12). A reduced form of folate, L-5-methyltetrahydrofolate (L-MTHF), which is stable in the supplemental form, has become available (13). L-MTHF is the pure crystalline synthetic derivative of the naturally occurring predominant form of folate (14) and may be more appropriate than folic acid as a fortificant, because it is unlikely to mask vitamin B-12 deficiency. Unlike folic acid, L-MTHF has to be converted to tetrahydrofolate (THF) via the vitamin B-12-dependent enzyme methionine synthase (EC 2.1.1.13) before it can participate in other folate-dependent reactions, including those that are essential for normal erythropoiesis (11, 12). When vitamin B-12 is deficient, L-MTHF is not converted to tetrahydrofolate and thus is not able to ameliorate megaloblastic anemia.

Before L-MTHF can be recommended as a potential fortificant, its effectiveness in lowering tHcy concentrations must be assessed against that of folic acid. Here we report the results of

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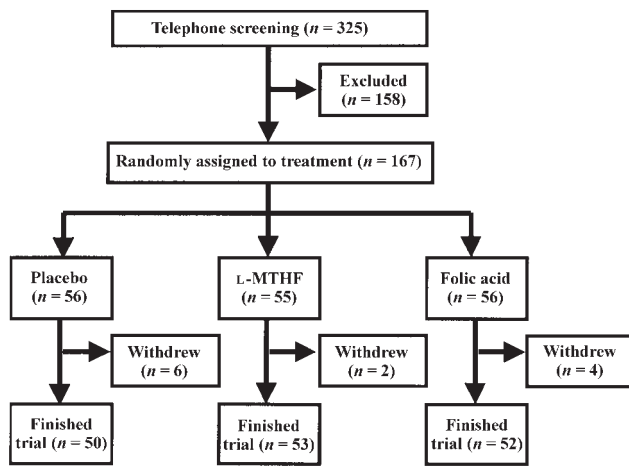


FIGURE 1. Participant flow and follow-up. L-MTHF, L-5-methyltetrahydrofolate.

a 24-wk, randomized, placebo-controlled trial designed to compare the efficacy of 100 µg folic acid/d with that of an equimolar amount of L-MTHF.

SUBJECTS AND METHODS

Subjects

Participants were recruited in March and April 2001 from Dunedin and Balclutha, New Zealand, through advertisement in local newspapers and the distribution of leaflets to homes. Respondents were invited to take part if they were aged ≥ 18 y and were neither users of supplements containing folic acid nor regular consumers of foods fortified with folic acid (ie, they ate <3 servings/wk). Respondents were not eligible if they had been diagnosed as having chronic disease, were pregnant, or were planning a pregnancy. The University of Otago Human Ethics Committee approved the study.

Study design

A 24-wk, double-blind, randomized, placebo-controlled trial was conducted between 1 April and 1 November 2001. At baseline, participants were asked to attend an early-morning clinic at which blood samples were collected after an overnight fast. As they arrived, participants were assigned to 1 of 3 treatment groups (placebo, folic acid, or L-MTHF) according to a computer-generated randomized list. A demographic and lifestyle questionnaire and a 63-item food-frequency questionnaire (FFQ), which was designed to assess each subject's customary folate intake over the previous month, were completed. Supplements were provided in aluminum-foil blister packs sufficient to last for 1 mo, and a diary form was provided. Compliance was assessed by counting of returned supplements and by review of the completed diary forms. Participants returned at 4-wk intervals to give a blood sample, to return compliance forms and unused supplements, and to collect a new supply of supplements and forms.

Supplements

The supplements were manufactured as hard gelatin capsules containing a blend of magnesium stearate and microcrystalline

cellulose as a filler (placebo) and either 100 µg (227 nmol) folic acid or 113 µg (227 nmol) L-MTHF as calcium salt (Metafolin; Eprova, Schaffhausen, Switzerland). Supplements were coded so that neither the investigators nor the participants were aware of the contents. Supplements were tested and both forms of folate were found to be completely stable (100% recovery) over the period of the study.

Laboratory methods and dietary analysis

Blood samples for measurement of plasma folate, tHcy, lipids, and creatinine were collected in EDTA-treated tubes, placed on ice immediately, separated within 2 h by centrifugation ($2000 \times g$ for 10 min at 4 °C), and stored at -80 °C until they were analyzed. Plasma vitamin B-12, creatinine, lipid, and lipoprotein were measured at baseline. Plasma folate, red blood cell folate (RCF), and tHcy were measured at baseline and at weeks 8, 16, and 24. Hematocrit was measured in freshly collected blood with the use of a hematology analyzer (Cell Dyn 1200; Abbott Laboratories, Abbott Park, IL). Plasma folate and whole-blood folate concentrations were measured with the use of the microtiter technique described by O'Broin and Kelleher (15) with chloramphenicol-resistant *Lactobacillus casei* as the test microorganism. RCF was calculated from whole-blood folate by subtracting plasma folate and correcting for hematocrit. The interassay CV was 13.6% on the basis of repeated measurements of a pooled control. The total cholesterol concentrations in plasma and lipoprotein fractions were measured enzymatically with kits and calibrators (Boehringer Mannheim, Mannheim, Germany) on a Cobas Fara analyzer (Roche Diagnostics, Basel, Switzerland), with a between-run CV of 0.9%. Plasma creatinine was measured enzymatically using Roche diagnostic kits on a Cobas Fara analyzer (CV 4.5%). Plasma tHcy and cobalamin (vitamin B-12) were determined on an Abbott IMx analyzer. The between-run CV for both assays was $<10\%$ on the basis of the controls provided by the manufacturer. Samples from each of the participants were tested in a single run to eliminate between-run variation. Identification of the 677C \rightarrow T polymorphism of the gene encoding for 5,10-methylenetetrahydrofolate reductase (EC 1.7.99.5) (MTHFR) was conducted as previously described (16). The New Zealand food-composition database (17) was used to determine the folate content of foods in the FFQ.

Statistical analysis

Where appropriate, data were log transformed to normalize the distribution and the estimates were back-transformed to geometric means with 95% CIs. Baseline characteristics and compliance between treatment groups were compared with the use of a one-way analysis of variance for continuous variables and of chi-square analyses for categorical variables. Distribution of the MTHFR polymorphism between the groups was compared with Fisher's exact test. At 24 wk, multiple regression was used to estimate the differences between the placebo group and each treatment group after adjustment for baseline values (18). Differences between the treatment groups and the placebo group and between the treatment groups were compared with the use of a Bonferroni post hoc test to adjust for multiple comparisons. Results were considered significant at $P < 0.05$. All analyses were undertaken using SPSS software, version 10, for Macintosh (SPSS Inc, Chicago).

RESULTS

The flow of participants is shown in **Figure 1**. Of the 325 people who responded, 158 were excluded for regular consumption



TABLE 1Characteristics of study participants in each treatment group at baseline¹

Characteristic	Placebo group (n = 50)	L-MTHF group (n = 53)	Folic acid group (n = 52)
Age (y) ²	47 ± 13.5	41 ± 13.5	46 ± 16.7
Women [n (%)]	36 (72)	43 (81)	38 (73)
Plasma vitamin B-12 (pmol/L)	279 (249, 312) ³	256 (228, 287)	270 (239, 304)
Plasma total cholesterol (mmol/L)	5.8 (5.5, 6.1)	5.3 (5.1, 5.6)	5.5 (5.2, 5.8)
Plasma creatinine (μmol/L)	96 (92, 101)	95 (90, 100)	98 (94, 103)
Dietary folate (μg/d)	241 (215, 270)	244 (217, 275)	211 (182, 244)
<i>MTHFR</i> 677C→T (n)			
C/C	24	30	27
C/T	22	18	22
T/T	4	5	3

¹L-MTHF, synthetic L-5-methyltetrahydrofolate; MTHFR, methyltetrahydrofolate reductase. There were no significant differences among treatment groups.

²Arithmetic $\bar{x} \pm$ SD.

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

of folic acid, either as a supplement or in fortified foods, or because of self-reported chronic disease. The remaining 167 persons were randomly assigned to 1 of 3 groups to receive a daily capsule containing either 100 μg folic acid (the molar equivalent of L-MTHF) or placebo. Twelve persons subsequently withdrew: 2 cited possible adverse reactions (1 each from the placebo and folic acid groups), and the other 10 withdrew for personal reasons. Blood was collected at 4-wk intervals (± 2.7 d) with 96%, 96%, and 100% attendance at weeks 8, 16, and 24, respectively. Compliance was not significantly different between the treatment groups ($P = 0.52$): 95% of subjects consumed >90% of their supplements (5% consumed 76–89% of the supplements, and 48% consumed 100% of the supplements).

Baseline characteristics of the participants are shown in **Table 1**. There were no significant differences in any of the characteristics among the groups. Dietary folate intake, as assessed by a semi-quantitative FFQ, correlated with baseline plasma folate concentrations (Spearman's $r = 0.19$, $P = 0.020$). The main outcomes of the intervention are shown in **Table 2**. Compared with the placebo group, plasma tHcy was significantly lower in both the L-MTHF and folic acid groups at 24 wk ($P < 0.001$). The

mean reduction (95% CI) in plasma tHcy of 14.6% (9.3, 19.5%) in the L-MTHF group was significantly greater ($P = 0.045$) than the reduction seen in the folic acid group [9.3% (3.7, 14.6%)]. Plasma folate and RCF were significantly higher at 24 wk in both of the supplemented groups than it was in the placebo group ($P < 0.01$). After supplementation, there were no significant differences in plasma folate or RCF concentrations between the 2 supplemented groups ($P > 0.05$).

DISCUSSION

This is the first study to compare the effect of low-dose L-MTHF with that of folic acid (≈ 100 μg/d) on plasma tHcy and folate concentrations in a free-living healthy population. Our findings indicate that low-dose L-MTHF is as effective as folic acid—and possibly more effective over the long-term—in lowering tHcy concentrations in healthy persons. At 24 wk of supplementation, plasma tHcy was 14.6% and 9.3% lower in the L-MTHF and folic acid groups, respectively, than it was in the placebo group. At 24 wk of supplementation, plasma folate was 34% and 52% higher and RCF 23% and 31% higher in the L-MTHF and folic acid groups,

TABLE 2Plasma total homocysteine (tHcy), plasma folate, and red blood cell folate (RCF) concentrations in the intervention groups at each time point¹

Treatment	Baseline ²	Week 8	Week 16	Week 24	Percentage difference from baseline at week 24 ³
Plasma tHcy (μmol/L)					
Placebo (n = 50)	8.5 (8.0, 9.1)	8.8 (8.2, 9.4)	8.8 (8.2, 9.4)	8.5 (7.9, 9.1)	
L-MTHF (n = 53)	8.8 (8.0, 9.6)	8.3 (7.7, 9.1)	8.1 (7.4, 8.8)	7.4 (6.9, 8.0)	−14.6 (−9.3, −19.5) ⁴
Folic acid (n = 52)	8.4 (7.7, 9.1)	8.1 (7.5, 8.7)	7.8 (7.2, 8.4)	7.6 (7.1, 8.2)	−9.3 (−3.7, −14.6) ^{4,5}
Plasma folate (nmol/L)					
Placebo (n = 50)	19.7 (17.4, 22.3)	19.0 (16.4, 22.0)	18.5 (15.9, 21.5)	20.5 (17.6, 24.0)	
L-MTHF (n = 53)	17.5 (15.4, 20.0)	22.3 (19.7, 25.2)	23.0 (19.8, 26.7)	25.6 (22.6, 28.9)	34 (14, 56) ⁴
Folic acid (n = 52)	23.3 (20.5, 26.5)	28.9 (25.8, 32.4)	28.5 (24.6, 33.1)	34.5 (30.5, 39.0)	52 (30, 78) ⁴
RCF (nmol/L)					
Placebo (n = 50)	884 (804, 972)	866 (781, 959)	884 (789, 991)	848 (752, 956)	
L-MTHF (n = 53)	814 (739, 897)	899 (822, 983)	1003 (926, 1087)	984 (910, 1064)	23 (12, 35) ⁴
Folic acid (n = 52)	915 (838, 999)	999 (924, 1079)	1057 (959, 1164)	1137 (1053, 1227)	31 (19, 44) ⁴

¹Geometric \bar{x} ; 95% CIs in parentheses. L-MTHF, L-5-methyltetrahydrofolate.

²There were no significant differences among the 3 groups at baseline.

³Relative to the placebo group.

⁴Significantly different from placebo group after adjustment for baseline, $P < 0.01$ (Bonferroni adjusted for multiple comparisons).

⁵Significantly different from the L-MTHF group after adjustment for baseline, $P < 0.05$ (Bonferroni adjusted for multiple comparisons).

respectively, than they were in the placebo group. At 24 wk, the increases in plasma folate and RCF concentrations did not differ significantly between the 2 supplemented groups.

A previous study compared racemic (D,L) methyltetrahydrofolate with folic acid by using doses of 480 and 400 μg , respectively (19). A reduction of 13% in tHcy after the ingestion of 400 μg folic acid/d over 8 wk compares favorably with a reduction of 9.3% in our study after the ingestion of 100 μg folic acid/d over 24 wk. In contrast, tHcy and plasma folate responded very differently to D,L-MTHF and L-MTHF in our study. Plasma tHcy declined by 3% and plasma folate increased 5-fold (431%) with the use of D,L-MTHF, compared with a 14.6% reduction in tHcy and a 34% increase in plasma folate with the use of L-MTHF. These differences may be explained by the different forms of methyltetrahydrofolate (D,L-MTHF and L-MTHF, respectively) used in the 2 studies (19). Only one-half of the 480 μg D,L-MTHF is the physiologically active L-isomer. Nevertheless, 240 μg is considerably more than the dose of L-MTHF used in our study. It is possible that the inactive D-isomer competes with the active L-isomer for cellular uptake but is unable to participate in the remethylation of homocysteine. The reason for the large increase (431%) in plasma folate concentrations in the study using D,L-MTHF supplementation is not known. Those authors suggested that the increase in plasma folate with the use of D,L-MTHF might be due to methodologic problems with the immunoassay that are associated with measuring of the D-isomer. Indeed, in a subsample of subjects, the concentrations of plasma folate were analyzed by the use of a combination HPLC and microbiologic method, and the results were compared with those obtained from the immunoassay. The apparent 5-fold increase in plasma folate concentration in their subjects found with the immunoassay was not found when the same samples were analyzed with the combined HPLC and microbiologic method.


A potential benefit of folate fortification in the United States has been an associated decline in tHcy concentrations in the population (10). Most but not all observational studies have indicated that an elevated tHcy concentration is an independent risk factor for occlusive vascular disease (20, 21). Recently, a reduction in restenosis rates from 39% to 26% after coronary angioplasty was shown in patients who took a daily folic acid, vitamin B-12, and vitamin B-6 supplement; this reduction was accompanied by a reduction in tHcy (9). Folic acid also improves the endothelial function independent of changes in tHcy (8), and restoration of endothelial function has been shown with the use of an infusion of methyltetrahydrofolate (22). Thus, given the similar effects of both folic acid and L-MTHF on endothelial function and a reduction of the tHcy concentration, it seems probable that any clinical benefit associated with folic acid is also likely to accrue from the use of L-MTHF.

The absolute (0.8–1.4 μmol) and proportional (9–15%) reductions in tHcy reported here with the use of either folic acid or L-MTHF are similar to the findings in other supplementation studies. The Homocysteine Lowering Trialists' Collaboration (23) suggested that supplementation with 0.5–5 mg folic acid/d reduces serum tHcy concentrations by 25%. In that meta-analysis, the median baseline tHcy was 11.8 $\mu\text{mol/L}$, whereas participants in the present study had a mean baseline tHcy of 8.5 $\mu\text{mol/L}$. It is clear that, the higher the baseline tHcy, the greater the decline with supplementation (23). Indeed, within our study, the tHcy-lowering

effect of either L-MTHF or folic acid was most pronounced in those subjects with the highest baseline tHcy concentrations. Participants whose baseline plasma tHcy was in the highest third had a 14% and 20% decline in tHcy concentration after 24 wk supplementation with folic acid and L-MTHF, respectively, and these results are comparable to those obtained in the meta-analysis.

It is conceivable that a higher dose of L-MTHF might have reduced tHcy to a greater extent. We elected to examine the effects of 100 μg L-MTHF/d, because a previous study using folic acid-fortified breakfast cereal showed reductions in tHcy of 18% over a 4-wk period, independent of doses that ranged from 100 to 300 μg folic acid/d (16). Persons with vascular disease may require higher doses for maximal reductions in tHcy (24).

Although lowering tHcy is potentially beneficial in reducing the risk of occlusive vascular disease, the main reason for the implementation of mandatory food fortification was to provide protection against NTD. The randomized controlled trials have used supplements containing folic acid (25–27). Nevertheless, it seems likely that the protection against NTD afforded by folic acid is due to an improved maternal folate status (28) rather than to the form of the vitamin per se. In a case-control study of 56 049 women in Ireland, the risk of NTD declined continuously as the RCF or serum folate concentration increased (28). A reduction in NTD risk, therefore, could be expected, given the 34% increase in plasma folate concentration and the 23% increase in RCF concentration found with low-dose L-MTHF supplementation reported here.

A potential advantage of L-MTHF over folic acid is that L-MTHF is unlikely to mask the hematologic signs of vitamin B-12 deficiency even at high intakes. Furthermore, L-MTHF does not require reduction by dihydrofolate reductase before being incorporated into the active cellular folate pool (29). In summary, we have shown that plasma tHcy concentrations are lowered and plasma folate and RCF concentrations are elevated by low-dose L-MTHF. Further studies of the effects of L-MTHF on homocysteine are required to confirm these findings, but consideration could be given now to L-MTHF as an alternative to folic acid for food fortification. 

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BV and TG had the original idea for the study; JM and BV obtained funds for the project; BV, TG, and RM were responsible for planning the study; and BV and TG recruited subjects and were responsible for sample collection, laboratory analysis, and statistical analysis. All authors contributed to the writing of the report. RM is the Chief Scientific Officer for Eprova AG, Switzerland, the manufacturer of Metafolin. RM participated in planning the study (supplement manufacture, dosage recommendation, supplement stability) and in writing the manuscript, but he was not involved in analysis or publication decisions. BV, TG, and JM had no conflict of interest.

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