

Human Nutrition and Metabolism Research Communication

Increases in Blood Folate Indices Are Similar in Women of Childbearing Age Supplemented with [6S]-5-Methyltetrahydrofolate and Folic Acid¹

(Manuscript received 4 July 2002. Initial review completed 5 August 2002. Revision accepted 18 August 2002.)

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ABSTRACT The natural diastereoisomer [6S]-5-methyltetrahydrofolate ([6S]-5-MTHF) may be a safer fortificant than folic acid for neural tube defect (NTD) prevention because it is unlikely to mask vitamin B-12 deficiency. An inverse relationship between NTD risk and blood folate concentrations has been reported. In this randomized, placebo-controlled, double-blind trial, we compared the effects of [6S]-5-MTHF and folic acid supplementation for 24 wk on plasma folate and red cell folate (RCF) in women of childbearing age (18–49 y). Women ($n = 104$) were randomly assigned to receive a supplement containing [6S]-5-MTHF (113 $\mu\text{g}/\text{d}$), folic acid (100 $\mu\text{g}/\text{d}$) or placebo. The mean estimated linear increase in plasma folate concentration was 0.3 [95% confidence interval (CI): 0.1, 0.5], and 0.4 (0.2, 0.6) nmol/(L · wk) in the [6S]-5-MTHF and folic acid groups, respectively. The mean estimated linear increase in RCF was 7.4 (95% CI: 4.5, 10.3), and 8.3 (4.4, 12.3) nmol/(L · wk) in the [6S]-5-MTHF and folic acid groups, respectively. There were no differences in the slopes between the [6S]-5-MTHF group and the folic acid group in either plasma folate ($P = 0.48$) or RCF ($P = 0.70$). At 24 wk, estimated mean increases in plasma folate concentrations were 6.9 (95% CI: 1.7, 12.2) and 9.2 (3.3, 15.1) nmol/L, and in RCF, 251 (143, 360) and 275 (148, 402) nmol/L, in the [6S]-5-MTHF and folic acid groups, respectively, relative to the placebo group. **These data suggest that low dose [6S]-5-MTHF and folic acid supplementation increase blood folate indices to a similar extent. A steady state in the blood indices had not been reached by 24 wk.** J. Nutr. 132: 3353–3355, 2002.

KEY WORDS: • [6S]-5-methyltetrahydrofolate supplementation
• folic acid supplementation • plasma folate
• red blood cell folate • women

Folic acid taken before and during early pregnancy reduces a woman's risk of a neural tube defect (NTD)³-affected pregnancy (1). A high rate of unplanned pregnancies led United States health authorities to mandate fortification of grain-based foods with folic acid in 1998 (140 $\mu\text{g}/100$ g enriched grains) (2). Mandatory fortification of foods has not occurred in many other countries in part because of concerns about excessive folic acid intakes. A major concern is that folic acid could mask the hematological signs of vitamin B-12 deficiency, delaying diagnosis and allowing progression of neurological damage (3,4). Recently, a reduced form of folate, [6S]-5-methyltetrahydrofolate ([6S]-5-MTHF), has become available that may be safer as a food fortificant because it is unlikely to mask a vitamin B-12 deficiency (5). Folic acid is able to mask the hematological signs of vitamin B-12 deficiency because it is readily converted to tetrahydrofolate, the form of folate that supports erythropoiesis, whereas [6S]-5-MTHF requires a vitamin B-12-dependent enzyme, methionine synthase, for conversion to tetrahydrofolate (3,4,6).

It is not known whether [6S]-5-MTHF will reduce the NTD rate. However, the risk of NTD is inversely associated with maternal plasma and red blood cell folate (RCF) concentrations (6). Accordingly, any strategy that increases blood folate could be expected to decrease NTD risk. Mandatory fortification of folic acid in the United States was estimated to increase average folic acid intake by women of childbearing age by 80–100 $\mu\text{g}/\text{d}$ (7). We compared the linear effects of three treatments (placebo; folic acid 100 $\mu\text{g}/\text{d}$; [6S]-5-MTHF 113 $\mu\text{g}/\text{d}$) over time on plasma folate and RCF in women of childbearing age (18–49 y).

SUBJECTS AND METHODS

Participants. Women of childbearing age ($n = 104$; 18–49 y old) were recruited through distribution of leaflets to homes and advertisement in local newspapers. Participants had no diagnosed chronic disease, were not pregnant or planning a pregnancy, and were not users of supplements or regular consumers of folic acid-fortified foods (<3 servings/wk). The Human Ethics Committee of the University of Otago approved the study and participants gave written informed consent.

Study design. The study was a 24-wk, placebo-controlled, double-blind trial. Participants were randomly assigned to one of three treatment groups, i.e., placebo, folic acid or an equimolar amount of [6S]-5-MTHF. Blood samples were taken from fasting subjects at baseline and at 4-wk intervals for 24 wk. Compliance was assessed by counting returned supplements and from completed diaries.

Supplements. The supplements were manufactured as hard gelatin capsules each 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) [6S]-5-methyltetrahydrofolic acid, calcium salt (Metafolin, Eprova, Schaffhausen, Switzerland). The natural diastereoisomer of 5-methyltetrahydrofolate has a [6S,

¹ Supported by The Otago Medical Research Foundation (Laurenson Foundation) and the Bristol Meyers Squibb Mead Johnson Award.

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³ Abbreviations used: CI, confidence interval; [6S]-5-MTHF, [6S]-5-methyltetrahydrofolate; NTD, neural tube defect; RCF, red blood cell folate.

α S] configuration, referred to in this paper as [6S]-5-MTHF. 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.

Biochemical analyses. Blood samples were collected in tubes containing EDTA. Plasma was separated within 2 h of collection by centrifugation ($2000 \times g$ for 10 min at 4°C). Plasma folate and whole-blood folate concentrations were determined using the micro-titer technique as described by O'Broin and Kelleher (8) with chloramphenicol resistant *Lactobacillus casei* as the test microorganism. RCF was calculated from whole-blood folate by subtracting plasma folate and correcting for hematocrit. Hematocrits were measured on freshly collected blood using a Cell Dyn 1200 (Abbott Laboratories, Abbott Park, IL). The interassay CV for the folate assay was 10.6% based upon repeated measurements of a pooled control.

Statistics. For RCF and plasma folate, a generalized estimating equation was fitted to test for significant differences between the linear effects of the treatments on the outcome over time. Each model included a separate intercept and slope term for each treatment group. The generalized estimating equation was fitted using the xtgee command in Stata 7.0 (College Station, TX), with an independent correlation structure and robust standard errors. This allowed for the correlations that occur within person from collecting multiple measurements from each participant over time.

RESULTS

Of the 104 women who started, 97 completed the trial and 7 withdrew. The age of the participants was 38 ± 8.4 y (mean \pm SD). The numbers of women in each group were placebo ($n = 28$), [6S]-5-MTHF ($n = 38$) and folic acid ($n = 31$). The RCF concentrations at baseline were 944 ± 364 , 837 ± 291 and 932 ± 311 nmol/L, and for plasma folate, 23.5 ± 10.8 , 20.0 ± 8.7 and 25.2 ± 11.5 nmol/L in the placebo, [6S]-5-MTHF and folic acid groups, respectively. There were no differences among the groups for baseline folate concentrations or age.

Over time, RCF and plasma folate concentrations increased in the folic acid and [6S]-5-MTHF groups (Fig. 1). The mean estimated increases in RCF per week were 7.4 [95% confidence interval (CI): 4.5, 10.3] and 8.3 (4.4, 12.3) nmol/(L \cdot wk) in the [6S]-5-MTHF and folic acid groups, respectively. The mean estimated increases per week were 0.3 (95% CI: 0.1, 0.5) and 0.4 (0.2, 0.6) nmol/(L \cdot wk) for plasma folate in the [6S]-5-MTHF and folic acid groups, respectively. In the placebo group, there were no changes over time in plasma folate 0.0 (95% CI: -0.1, 0.2) or RCF -3.1 (-6.6, 0.4) nmol/(L \cdot wk). There were no differences in the slopes between the [6S]-5-MTHF group and the folic acid group in either plasma ($P = 0.48$) or RCF ($P = 0.70$).

At 24 wk, estimated mean increases in plasma folate concentrations were 6.9 (95% CI: 1.7, 12.2) and 9.2 (3.3, 15.1) nmol/L, and in RCF, 253 (143, 360) and 275 (148, 402) nmol/L, in the [6S]-5-MTHF and folic acid groups, respectively, relative to the placebo group. The estimated mean differences in blood folate concentrations between the folic acid group and the [6S]-5-MTHF group at 24 wk were 2.3 nmol/L (95% CI: -4.0, 8.6) and 23 nmol/L (-95, 142) for plasma folate and RCF, respectively. There appeared to be no plateau reached by 24 wk.

DISCUSSION

We estimated that 24 wk of low dose folate supplementation ($\sim 100 \mu\text{g/d}$) raises mean RCF concentrations by 251–275 nmol/L and plasma folate concentrations by 6.9–9.2 nmol/L in

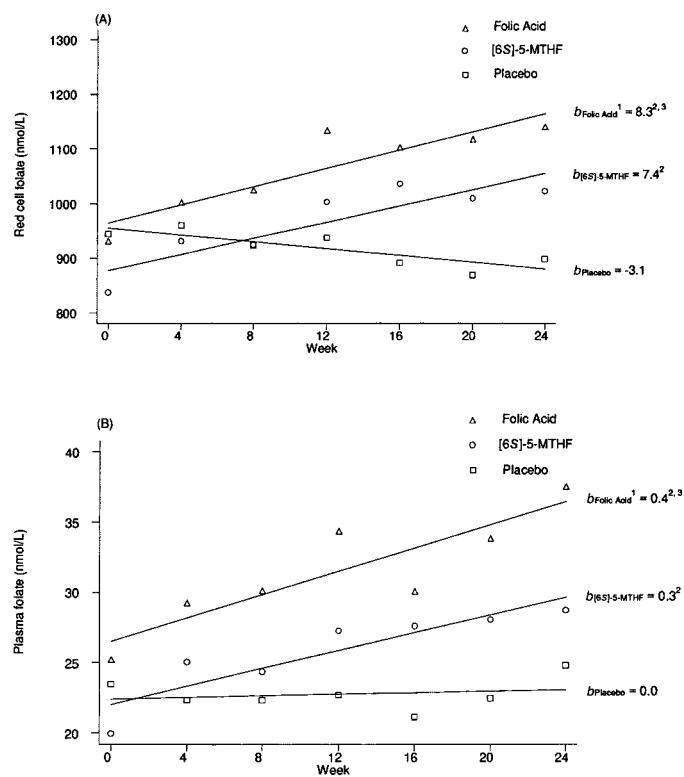


FIGURE 1 Weekly mean changes in red blood cell (A) and plasma (B) folate concentrations in women of childbearing age (18–49 y) supplemented with 113 $\mu\text{g/d}$ [6S]-5-methyltetrahydrofolate ([6S]-5-MTHF; $n = 38$); 100 $\mu\text{g/d}$ folic acid ($n = 31$); and placebo ($n = 28$). The fitted lines were determined from a generalized estimating equation. ¹ b represents the slope in nmol/(L \cdot wk). ²The treatment slope differed from the placebo slope ($P \leq 0.01$). ³Slopes did not differ between folic acid and [6S]-5-MTHF ($P > 0.05$).

women of childbearing age. Our increase in RCF was higher than that reported by Daly and co-workers (9) in which RCF folate increased by 180 nmol/L after 6 mo in women taking 100 μg folic acid/d. However, compliance may have been lower in their study. There are no other published reports on the effects of [6S]-5-MTHF on blood folate concentrations in women of childbearing age. Fohr and co-workers (10) compared the effects of racemic [6S, 6R]-5-MTHF (480 $\mu\text{g/d}$) with folic acid (400 $\mu\text{g/d}$) on plasma and erythrocyte folate over 8 wk. It is difficult to make meaningful comparisons between the two studies. Only half of racemic methyltetrahydrofolate is the physiologically active [6S]- isomer and the authors reported methodological problems associated with measuring folate by the immunoassay used due to possible interference by the [6R]-isomer.

An advantage of [6S]-5-MTHF over folic acid is that it is unlikely to mask a vitamin B-12 deficiency. Thus, [6S]-5-MTHF may be an attractive alternative to folic acid for the prevention of NTD. We recognize that it has not been established through randomized trials that [6S]-5-MTHF will reduce NTD rate. However, in a case control study in Ireland, risk of NTD was inversely associated with maternal plasma and RCF concentrations (6). Most of the women in the Irish cohort, even those with high blood folate concentrations, were probably not receiving folic acid. Thus, any strategy that increases blood folate could be expected to decrease NTD risk. Further, observational studies (11–13) and at least one intervention

study (14) suggest that intake of naturally occurring folate is inversely associated with NTD occurrence. Naturally occurring folates found in food are polyglutamylated and in a reduced form. Absorption of natural folates is usually considered to be less efficient than absorption of folic acid, possibly due to the food matrix or incomplete removal of polyglutamates by intestinal conjugase (15). Synthetic [6S]-5-MTHF monoglutamate in supplemental form is not food bound; it does not require conjugase activity and absorption characteristics are similar to those of folic acid (16). Accordingly, we would expect [6S]-5-MTHF monoglutamate to be at least as effective as dietary natural folates, which consist mainly of [6S]-5-MTHF polyglutamates, at decreasing NTD rates. The [6S]-5-MTHF used in this study (as a calcium salt) was completely stable in supplemental form over the study period. Preliminary tests by the manufacturer indicate good stability of [6S]-5-MTHF when sprayed onto cereals and incorporated into white bread. Stability of [6S]-5-MTHF in food processing operations would have to be confirmed if it were to be considered as a food fortificant.

Our analysis suggested that neither RCF nor plasma folate concentrations had reached a steady state at 24 wk. The results indicate that a longer duration is required to assess the maximal effect of folate supplementation on blood folate accumulation. Plasma folate concentration is considered a short-term indicator of folate intake, whereas RCF changes over a longer term, reflecting the erythrocyte lifespan of 120 d. Our finding that blood folate concentrations had not reached a plateau by 24 wk was unforeseen. In a preliminary study with a small number of subjects, Ward et al. (17) found that plasma folate concentrations had peaked by 6 wk for doses of folic acid <200 µg, whereas it took up to 14 wk for doses of 400 µg folic acid to stabilize. These findings have implications for women planning a pregnancy. Health authorities in several countries advise women to take folic acid before becoming pregnant (18). A question that remains is, how long before becoming pregnant should a woman take a supplement? Our findings suggest that the accumulation of blood folate was slower than previously thought. Accordingly, achieving a maximal increase in blood folate associated with NTD risk reduction may take several months.

In conclusion, we have shown that low dose [6S]-5-MTHF supplementation raises blood folate indices up to 24 wk. Further, blood folate indices had not reached a steady state at 24 wk in either of the folate-supplemented groups. On the basis of changes in blood folate indices, we would expect that [6S]-5-MTHF would be effective in decreasing NTD rate.

ACKNOWLEDGMENT

Eprova AG (Switzerland), an affiliate of Merck KGaA, Darmstadt, Germany, provided the supplements.

LITERATURE CITED

- Lumley, J., Watson, L., Watson, M. & Bower, C. (2000) Periconceptional supplementation with folate and/or multivitamins for preventing neural tube defects. [Retraction of Lumley, J., Watson, L., Watson, M. & Bower, C. Cochrane Database System Rev. 2001: CD001056; 11686974.] Cochrane Database System Rev. CD001056.
- Anonymous (1996) Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid. Fed. Regist. 61: 8781–8797.
- Scott, J. M. & Weir, D. G. (1981) 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* 2: 337–340.
- Weir, D. G. & Scott, J. M. (1999) Brain function in the elderly: role of vitamin B-12 and folate. *Br. Med. Bull.* 55: 669–682.
- Eprova AG. (2000) Metafolin: About the Product. Internet: <http://www.metafolin.com> accessed 18 September 2002.
- Daly, L. E., Kirke, P. N., Molloy, A., Weir, D. G. & Scott, J. M. (1995) Folate levels and neural tube defects: implications for prevention. *J. Am. Med. Assoc.* 274: 1698–1702.
- Yetley, E. A. & Rader, J. I. (1995) Folate fortification of cereal-grain products: FDA policies and actions. *Cereal Foods World* 40: 67–72.
- O'Broin, S. & Kelleher, B. (1992) Microbiological assay on microtitre plates of folate in serum and red cells. *J. Clin. Pathol.* 45: 344–347.
- Daly, S., Mills, J. L., Molloy, A. M., Conley, M., Lee, Y. J., Kirke, P. N., Weir, D. G. & Scott, J. M. (1997) Minimum effective dose of folic acid for food fortification to prevent neural-tube defects. *Lancet* 350: 1666–1669.
- Fohr, I. P., Prinz-Langenohl, R., Brönstrup, A., Bohlmann, A. M., Nau, H., Berthold, H. K. & Pietrzik, K. (2002) 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.* 75: 275–282.
- Milunsky, A., Jick, H., Jick, S. S., Bruell, C. L., MacLaughlin, D. S., Rothman, K. J. & Willett, W. (1989) Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. *J. Am. Med. Assoc.* 262: 2847–2852.
- Bower, C. & Stanley, F. J. (1989) Dietary folate as a risk factor for neural-tube defects: evidence from a case-control study in Western Australia. *Med. J. Aust.* 150: 613–619.
- Werler, M. M., Shapiro, S. & Mitchell, A. A. (1993) Periconceptional folic acid exposure and risk of occurrent neural tube defects. *J. Am. Med. Assoc.* 269: 1257–1261.
- Laurence, K. M., James, N., Miller, M. & Campbell, H. (1980) Increased risk of recurrence of pregnancies complicated by fetal neural tube defects in mothers receiving poor diets, and possible benefit of dietary counselling. *Br. Med. J.* 281: 1592–1594.
- Brouwer, I. A. & Van Dusseldorp, M. (2001) Bioavailability and bioefficacy of folate and folic acid in Man. *Nutr. Res. Rev.* 14: 267–293.
- Prinz-Langenohl, R., Fohr, I., Tobolski, O., Finglas, P. & Pietrzik, K. (2001) Bioavailability of 6S-5-methyltetrahydrofolate relative to folic acid. Bioavailability 2001, Interlaken, Switzerland.
- Ward, M., McNulty, H., McPartlin, J., Strain, J. J., Weir, D. G. & Scott, J. M. (1997) Plasma homocysteine, a risk factor for cardiovascular disease, is lowered by physiological doses of folic acid. *Q. J. Med.* 90: 519–524.
- Cornel, M. C. & Erickson, J. D. (1997) Comparison of national policies on periconceptional use of folic acid to prevent spina bifida and anencephaly (SBA). *Teratology* 55: 134–137.