Low-dose folic acid supplementation reduces plasma levels of the cardiovascular risk factor homocysteine in postmenopausal women

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OBJECTIVE: The aim of our randomized, controlled trial was to verify the effect of folic acid supplementation on homocysteine levels in postmenopausal women.

STUDY DESIGN: Thirty-six women were divided randomly into 2 groups as follows: a placebo group (n = 18) and a group receiving 500 μg folic acid per day for 4 weeks (n = 18). To assess concentrations of plasma homocysteine, venous blood samples were taken on enrollment and after 4 weeks of treatment.

RESULTS: Mean plasma homocysteine levels were 10.9 ± 2.7 μmol/L in the placebo group and 7.8 ± 2.35 μmol/L ($P < .01$) in the group receiving 500 μg folic acid per day for 4 weeks. The thirds (referred to as tertiles) of women with the highest baseline homocysteine plasma levels showed the greatest reduction in homocysteine, with a mean decrease of 4.35 μmol/L (32%; $P < .01$), in comparison with a decrease of 3.35 μmol/L (29%; $P < .01$) in the middle tertile and 1.3 μmol/L (22.4%; $P = .09$) in the lower tertile.

CONCLUSIONS: The results show that low doses of folic acid are associated with a significant reduction in plasma concentrations of homocysteine. The highest initial levels of homocysteine showed the most important reduction after therapy. (Am J Obstet Gynecol 2000;183:945-7.)

Key words: Folic acid, cardiovascular risk, homocysteine, postmenopausal, women

Cardiovascular diseases are the main cause of death, morbidity, and disability in women of Western countries.[1] The incidence of cardiovascular disease is lower in women younger than 50 years than in men of similar age but increases with age.[2]

Elevated plasma levels of the sulfur amino acid homocysteine are a risk factor for cardiovascular disease.[3] Even moderate homocystinemia is associated with an increased risk of premature cardiovascular disease.[4]

Changes in endothelial function may be at least partly induced by derangement of homocysteine metabolism. High blood levels of homocysteine have been reported in postmenopausal women.[5] Homocysteine interferes with platelet-endothelium interaction by reducing the production of nitric oxide and prostacyclin and promotes clotting by enhancing endothelial factor V activity and reducing the activity of thrombomodulin.[6-8]

There are indications that plasma homocysteine is related to estrogen status. Premenopausal women have lower plasma homocysteine levels than do men and postmenopausal women,[9] and plasma homocysteine levels are decreased in menopausal women receiving estrogen replacement therapy.[10]

Plasma homocysteine levels are partly genetically determined,[11] but acquired states such as cobalamin and folate deficiencies and renal failure may increase the levels. Folate (as methyltetrahydrofolate) is required for methylation of homocysteine to methionine catalyzed by methionine synthase with vitamin B_{12} as cofactor. Blood levels of homocysteine are inversely related to blood levels of folate, vitamin B_{12}, and vitamin B_6.[12]

Several randomized, controlled trials have shown that dietary supplements of folic acid lower homocysteine levels.[13, 14]

The aim of our randomized, controlled trial was to ver-

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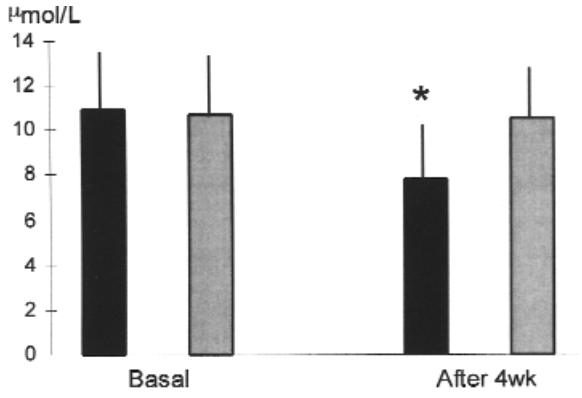

Fig 1. Plasma homocysteine levels are significantly reduced in postmenopausal women after 4 weeks of folic acid supplementation. *Dark bars,* Folic acid group; *shaded bars,* placebo group; *asterisk,* $P < .01$, versus baseline and placebo group.

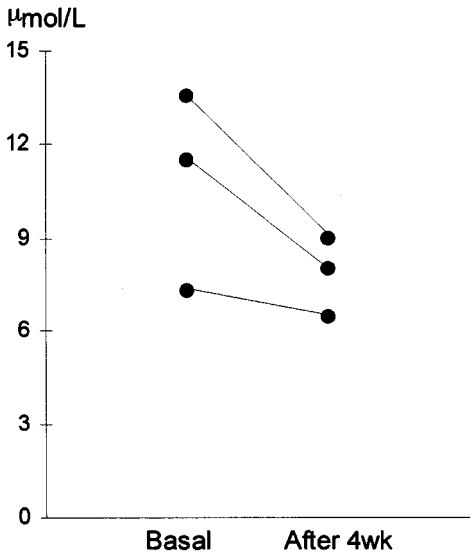

Fig 2. Tertiles of women with highest baseline plasma homocysteine levels showed most important reduction in homocysteine.

ify the effect of folic acid supplementation on homocysteine plasma levels in postmenopausal women.

Material and methods

In a recent meta-analysis it emerged that the effect of folic acid supplementation on homocysteine levels was similar for daily doses ranging from 0.5 to 5 mg daily.[13] We therefore decided to use the lowest dose (500 μg/d).

Fifty healthy women, aged 54 to 58 years, were selected, but only 36 completed the trial. Fourteen women were excluded from the study and were considered to be dropouts because therapy was interrupted or because they did not come for the blood examination. All had a menopausal status of at least 2 years' duration. Smokers and women with gastrointestinal disorders were excluded. None of the women had past or present renal or vascular disease, and none were taking medication or vitamin supplements. All had normal plasma folate concentrations. For all the subjects, protein and albumin levels were in the normal range. None of the women were receiving hormone replacement therapy. The study was approved by the institutional review board of the University of Siena. Written informed consent was obtained from each subject. There were no differences in the characteristics of the women who completed the trial and the women who did not.

The women were randomly assigned (according to a random number table) to blind treatment with placebo (n = 18) or 500 μg folic acid per day (n = 18). Subjects were instructed to maintain their regular diet and lifestyle. To assess concentrations of plasma homocysteine, we took venous blood samples on enrollment and after 4 weeks of treatment. Blood samples were obtained between 8 and 10 AM, after 12 hours of fasting. Compliance was assessed by interview at the end of the treatment.

Assay. Three milliliters of whole blood was collected in a Vacutainer (Becton Dickinson, Rutherford, NJ) blood collecting tube containing ethylenediaminetetraacetic acid cooled on ice and immediately centrifuged at 1000*g* for 5 minutes at 4°C.

The total homocysteine level was determined by high-performance liquid chromatography and fluorescence detection according to the method of Araki and Sako.[15]

High-performance liquid chromatography was performed with a Kontron model 320 (Kontron Instruments, Milan, Italy) system, and the fluorescence intensities were measured with a Kontron SFM 25 (Kontron Instruments) fluorescence spectrophotometer.

Statistical analysis. The results were expressed as mean ± SD. Plasma homocysteine levels measured before and after treatment were compared by means of the Wilcoxon rank test. A P value < .01 was considered statistically significant.

Results

The parameters of the 2 groups of women were similar at entry. There were no significant differences in age at entry (56 ± 1.4 vs 55.8 ± 1.5 years), age at menopause (49.1 ± 1.9 vs 48.9 ± 2.1 years), body mass index (27.8 ± 2.5 vs 28 ± 1.9 [Weight in kilograms/Height in centimeters^2]), and mean basal homocysteine levels (10.9 ± 2.7 vs 10.7 ± 2.5 μmol/L).

Mean plasma homocysteine levels were 10.9 ± 2.7 μmol/L in the placebo group and 7.8 ± 2.35 μmol/L in the group receiving 500 μg folic acid per day for 4 weeks (Fig 1). This difference was significant (27.8%) ($P < .01$).

The thirds (referred to as tertiles) of women with the highest baseline homocysteine plasma levels showed the greatest reduction in homocysteine (Fig 2), with a mean

decrease of 4.35 μmol/L (32%; $P < .01$), compared with a decrease of 3.35 μmol/L (29%; $P < .01$) in the middle tertile and 1.3 μmol/L (22.4%; $P = .09$) in the lower tertile.

Comment

To our knowledge, this is the first randomized, placebo-controlled trial of the effect of folic acid supplements on plasma concentrations of homocysteine in postmenopausal women. The only other study on this question, published 10 years ago, sampled 5 menopausal women treated with folic acid 5 mg/d; homocysteine was assayed with a JEOL (JEOL USA, Inc, Peabody, Mass) amino acid analyzer (model JLC-6AH) rather than with high-performance liquid chromatography.[16] Our results show that low doses of folic acid are associated with a significant reduction in plasma concentrations of homocysteine. The highest initial levels of homocysteine showed the most important reduction after therapy. In the group of women with lower basal levels of homocysteine, the reduction achieved after folic acid supplementation lost its significance ($P = .09$).

Homocysteine is an independent risk factor for premature vascular disorders, including coronary artery disease,[17] cerebrovascular disease,[18] and peripheral vascular disease.[19] The results of a prospective study of US physicians[4] suggest that moderate homocyst(e)inemia is associated with an increased risk of premature vascular disorders. The subjects who subsequently had myocardial infarction previously had significantly higher basal plasma homocysteine levels than did control subjects matched for age and smoking habits.

In homocystinuria, which is a rare inherited disorder, plasma homocysteine levels are markedly elevated (>50 μmol/L) and patients have severe vascular disease. Methionine taken orally is converted to homocysteine by demethylation, and the effect of an oral load can be used as a diagnostic test to identify individuals with enzyme defects who show an exaggerated rise in homocysteine levels.[20]

The importance of reducing plasma concentrations of homocysteine in postmenopausal women is indicated by the finding that prolonged lowering of homocysteine levels by 1 μmol/L is associated with approximately a 10% reduction in the risk of vascular disease and a reduction of 3 to 4 μmol/L is associated with a risk reduction of 30% to 40%.[21]

The data showing that low doses of folic acid reduce plasma concentrations of homocysteine pave the way for the fortification of foods with low doses of folic acid so as to reduce plasma levels of this amino acid. Proposals to fortify flours and cereal products with folate to reduce the incidence of neural tube defects have been discussed by the US Food and Drug Administration.[21] It has been estimated that in the United States fortification at 350 μg/100 g would reduce deaths per year from coronary artery disease by 30,000 in men and 19,000 in women.[22]

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