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REDUCTION OF PLASMA HOMOCYST(E)INE LEVELS BY BREAKFAST CEREAL FORTIFIED WITH FOLIC ACID IN PATIENTS WITH CORONARY HEART DISEASE

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ABSTRACT

Background The Food and Drug Administration (FDA) has recommended that cereal-grain products be fortified with folic acid to prevent congenital neural-tube defects. Since folic acid supplementation reduces levels of plasma homocyst(e)ine, or plasma total homocysteine, which are frequently elevated in arterial occlusive disease, we hypothesized that folic acid fortification might reduce plasma homocyst(e)ine levels.

Methods To test this hypothesis, we assessed the effects of breakfast cereals fortified with three levels of folic acid, and also containing the recommended dietary allowances of vitamins B₆ and B₁₂, in a randomized, double-blind, placebo-controlled, crossover trial in 75 men and women with coronary artery disease.

Results Plasma folic acid increased and plasma homocyst(e)ine decreased proportionately with the folic acid content of the breakfast cereal. Cereal providing 127 μ g of folic acid daily, approximating the increased daily intake that may result from the FDA's enrichment policy, increased plasma folic acid by 30.8 percent ($P=0.045$) but decreased plasma homocyst(e)ine by only 3.7 percent ($P=0.24$). However, cereals providing 499 and 665 μ g of folic acid daily increased plasma folic acid by 64.8 percent ($P<0.001$) and 105.7 percent ($P=0.001$), respectively, and decreased plasma homocyst(e)ine by 11.0 percent ($P<0.001$) and 14.0 percent ($P=0.001$), respectively.

Conclusions Cereal fortified with folic acid has the potential to increase plasma folic acid levels and reduce plasma homocyst(e)ine levels. Further clinical trials are required to determine whether folic acid fortification may prevent vascular disease. Until then, our results suggest that folic acid fortification at levels higher than that recommended by the FDA may be warranted. (N Engl J Med 1998;338:1009-15.)

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AFTER Hibbard and Smithells suggested a possible association between low folic acid levels and congenital neural-tube defects,¹ seven subsequent epidemiologic studies supported the proposition that periconceptual folic acid supplementation may reduce the occurrence of neural-tube defects.² Moreover, results of double-blind trials of primary³ and secondary⁴ prevention demonstrated that periconceptual daily intake of 0.8 mg and 4 mg of folic acid, respectively, reduced the incidence of neural-tube defects. The intake of folic acid derived from food by women of childbearing potential in the United States may be as low as 110 to 140 μ g per day,⁵ which is well below the recommended dietary allowance (RDA) of 200 μ g per day.⁶ Consequently, as of January 1 of this year, cereal-grain products in the U.S. food supply are being fortified with folic acid to prevent neural-tube defects.^{5,7-9}

It has been estimated that the level of folic acid fortification recommended by the Food and Drug Administration (FDA) (140 μ g per 100 g of cereal-grain products) would increase folic acid intake by 80 to 100 μ g per day in women of childbearing potential and by 70 to 120 μ g per day in adults older than 50 years.⁵ Since folic acid supplementation reduces levels of plasma homocyst(e)ine, or plasma total homocysteine,¹⁰⁻¹³ which may be elevated in 13 to 47 percent of patients with arterial occlusive diseases,¹⁴⁻¹⁸ we hypothesized that nationwide fortifica-

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tion of food with folic acid might reduce plasma homocyst(e)ine levels. To test this hypothesis in patients with coronary artery disease, we assessed the effects of breakfast cereals fortified with three levels of folic acid and the RDAs of certain vitamins, including B₆ and B₁₂.

METHODS

Subjects

The subjects were recruited from cardiology and primary care clinics associated with Providence St. Vincent Medical Center, in Portland, Oregon, and from a cohort of patients discharged from Providence St. Vincent Hospital with a diagnosis of ischemic heart disease (*International Classification of Diseases, 9th Revision*, codes 410 through 414). The study population included 81 unrelated white men and women, 45 to 85 years of age at the time of the initial interview. Subjects were excluded if they had a history of wheat intolerance or were taking medications that may influence plasma homocyst(e)ine levels (e.g., methotrexate, anti-convulsant agents, bile acid sequestrants, folic acid, or multivitamins). Two subjects were excluded because of misinformation regarding previous medications, and four dropped out after having a stroke (one subject), having rectal bleeding (one), not being able to attend scheduled appointments (one), and "feeling ill" during the washout period (one). Two subjects who answered "unknown" to the question of whether they had a history of diabetes or hypertension were included. Subjects were advised to

continue with their regular medications and diets, except for eating the breakfast cereal provided (see below) throughout the trial. The study was approved by the institutional review boards of Providence St. Vincent Medical Center and the Oregon Regional Primate Research Center.

The subjects had been found more than three months previously to have histories of acute myocardial infarction, angina pectoris documented by a cardiologist, percutaneous transluminal coronary angioplasty, or coronary-artery bypass graft surgery. The subjects reported having no history of stroke, intermittent claudication, or peripheral arterial revascularization.

Study Design

All the participants signed an informed-consent form, completed a medical-history form, and were randomly assigned to one of three groups (groups A, B, and C) for entry into a double-blind, placebo-controlled, crossover trial (Fig. 1). Thirty-gram packets of wheat-based, ready-to-eat cereals were prepared by the food-research laboratories of General Mills (Minneapolis). All the cereals contained, except as noted, the RDAs of the vitamins and minerals listed in the legend to Figure 1. Three experimental cereals were used; the mean (\pm SD) folic acid content per 30 g of cereal was $127 \pm 11 \mu\text{g}$ in the first cereal, $499 \pm 6 \mu\text{g}$ in the second (Total, General Mills), and $665 \pm 48 \mu\text{g}$ in the third; they all contained pyridoxine ($1.8 \pm 0.1 \text{ mg}$ per 30 g) and cyanocobalamin ($6.1 \pm 0.3 \mu\text{g}$ per 30 g). The placebo consisted of breakfast cereal without added folic acid, pyridoxine, or cyanocobalamin; the naturally occurring quantities of these vitamins were 10 ± 0.9 , 0.11 ± 0.02 , and $0.13 \pm 0.02 \mu\text{g}$ per 30 g of cereal, respectively.

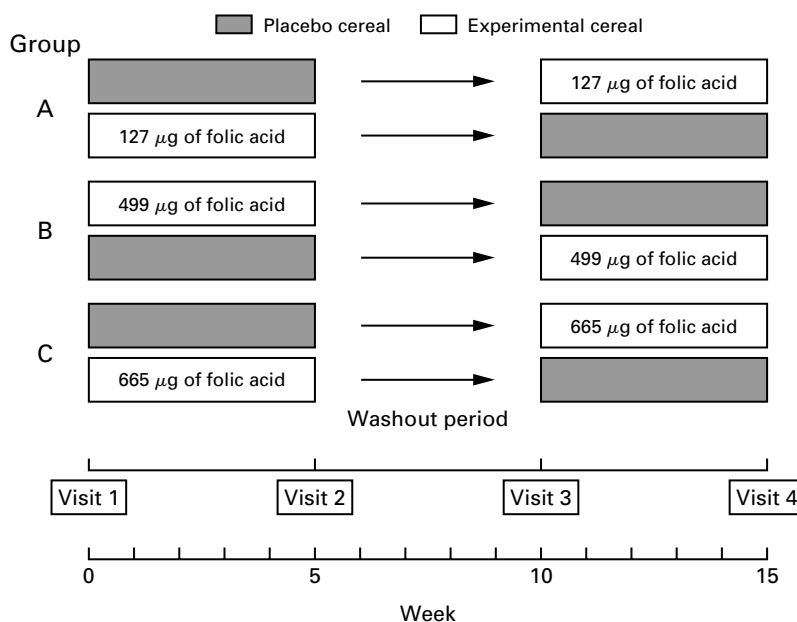


Figure 1. Diagram of Experimental Design.

Subjects were randomly assigned to receive 30 g of experimental or placebo cereal daily during the initial and final five-week periods. All daily portions of cereals contained the RDAs of vitamins C, E, B₁, B₂, B₃, B₅, B₆, B₁₂, iron, and zinc; 25 percent of the RDA of vitamin A; and 10 percent of the RDA of vitamin D. Three levels of folic acid fortification were used in the experimental cereals; the mean (\pm SD) folic acid content per 30 g of cereal was $127 \pm 11 \mu\text{g}$ (group A), $499 \pm 6 \mu\text{g}$ (group B), or $665 \pm 48 \mu\text{g}$ (group C). The placebo cereal contained the above-mentioned vitamins and minerals, except for folic acid and vitamins B₆ and B₁₂. The naturally occurring amounts of these vitamins were 10 ± 0.9 , 0.11 ± 0.02 , and $0.13 \pm 0.02 \mu\text{g}$ per 30 g of cereal, respectively. Basal plasma levels of homocyst(e)ine and vitamins were measured at visits 1 and 3; follow-up levels were measured at visits 2 and 4.

Each subject was requested to eat one 30-g packet of breakfast cereal daily for five weeks, followed by a five-week "washout" period during which the subjects consumed their usual diet. After this, they were to eat one packet of breakfast cereal daily for another five weeks (Fig. 1). The subjects were randomly assigned to eat either cereal containing one of the three levels of folic acid fortification or the placebo cereal for the initial five weeks and the alternate cereal during weeks 10 to 15. The experimental and placebo cereals replaced other breakfast cereals eaten by the participants before entering the study. During the second and fourth visits, all the remaining packets of cereal were returned and counted for an assessment of compliance.

Laboratory Analyses

Venous blood from fasting subjects was drawn into Vacutainer tubes containing EDTA. Plasma was separated within 30 minutes in a refrigerated centrifuge at 4°C and frozen at -20°C for analysis of homocyst(e)ine levels by high-performance liquid chromatography and electrochemical detection as described elsewhere,^{19,20} with minor modifications (interassay coefficient of variation, 9.1 percent; intraassay coefficient of variation, 5.0 percent). Samples from all four visits were analyzed simultaneously. The plasma homocyst(e)ine level is the sum of the homocysteine and homocysteinyl moieties of the disulfides homocystine and homocysteine-cysteine, whether free or bound to proteins. Hyperhomocyst(e)inemia is defined as a level of homocyst(e)ine in plasma or serum that is more than 2 SD above the mean value in control groups. Additional plasma aliquots were protected from light and frozen at -20°C for radioimmunoassay of folic acid (coefficient of variation, 7.8 percent) and vitamin B₁₂ (coefficient of variation, 5.4 percent) (Bio-Rad Quantaphase II, Bio-Rad Diagnostics, Hercules, Calif.) and for radioenzymatic assay of pyridoxal 5'-phosphate (coefficient of variation, 14.4 percent) (American Laboratory Product, Windham, N.H., and Bühlman Laboratories, Schönenbuch, Switzerland). Eleven samples with pyridoxal 5'-phosphate values ≥83.6 ng per milliliter (500 nmol per liter) were diluted and reanalyzed.

Blood cells were frozen at -20°C for analysis of the C677T methylenetetrahydrofolate reductase polymorphism, as described elsewhere.²¹ The amplification-reaction mixture was subjected to 30 cycles of amplification at 94°C for 30 seconds, 62°C for 30 seconds, and 72°C for 30 seconds. The polymerase-chain-reaction products were precipitated with ethanol and digested overnight with *Hinf*I (New England Biolabs, Beverly, Mass.); DNA fragments were analyzed by 3 percent agarose-gel electrophoresis.

Twenty packets of the placebo breakfast cereal and of each of the three folic acid-fortified cereals were analyzed for the content of folic acid and vitamins B₆ and B₁₂ with the use of microbiologic assays²² (General Mills). The vitamin composition was coded, and the study was conducted under double-blind conditions until the code was broken for interim and final statistical analyses. The plasma levels of homocyst(e)ine and of the vitamins were quantified without knowledge of the experimental groups or interactions.

Statistical Analysis

The distribution of study variables was examined with the use of standard exploratory analytical techniques for independent subjects. Paired data (placebo vs. experimental) in each group (A, B, and C) were analyzed separately. Basal and follow-up values were measured at the beginning and end of each treatment phase, respectively. Changes in homocyst(e)ine and vitamin levels associated with the intake of breakfast cereals were calculated as absolute and percent changes. The absolute change was calculated as the difference between the plasma levels at the end of the experimental phase and those at the end of the placebo phase; these data were used for correlation analyses. The percent change was calculated as the absolute change divided by the follow-up values after the placebo phase, with the result multiplied by 100; these data were used for intragroup and intergroup comparisons. Log-

arithmic transformations of the variables were performed as needed to improve normality. Adjustment for potential covariates was carried out by using multiple linear and stepwise regression. Correlations used Pearson's product moment for parametric data and Spearman's rank-order test for nonparametric data. The P values are two-sided. The statistical analyses were conducted with Excel (Microsoft, Redmond, Wash.), SigmaStat (Jandel Scientific, San Rafael, Calif.), Statmate (GraphPad Software, San Diego, Calif.), and Stat 100 (Biosoft, Ferguson, Mo.) software.

RESULTS

Characteristics of the Subjects

The characteristics of the three groups of subjects at entry (Table 1) were similar, although the number of women in group A was larger than in groups B and C. Mean basal homocyst(e)ine levels were lower in the 16 women (9.1 μmol per liter; 95 percent confidence interval, 7.8 to 10.5) than in the 59 men (13.1 μmol per liter; 95 percent confidence interval, 10.8 to 15.4) (P<0.001 by the Mann-Whitney U test). No other variables, including responses to the experimental cereals, were significantly different in the men and the women. Low levels of plasma folic acid were not overrepresented in our study population, since mean plasma folic acid levels at base line were similar to control data from 191 subjects without coronary heart disease from Portland, Oregon, who did not

TABLE 1. CHARACTERISTICS OF THE SUBJECTS AT ENTRY, ACCORDING TO STUDY GROUP.*

CHARACTERISTIC	ALL SUBJECTS	GROUP A (N=24)	GROUP B (N=25)	GROUP C (N=26)
Mean folic acid — μg per 30 g of cereal	—	127	499	665
Age — yr	64±10	64±11	66±8	63±12
Body-mass index†	27.9±4.3	28.5±5.1	28.8±6.1	27.5±4.4
History of diabetes — no. (%)‡	20 (27)	9 (38)	4 (16)	7 (27)
History of hypertension — no. (%)‡	42 (57)	14 (58)	13 (52)	15 (58)
Male sex — no. (%)	59 (79)	13 (54)	23 (92)§	23 (88)§
Previously ate breakfast cereal — no. (%)‡¶	36 (49)	10 (42)	15 (60)	11 (42)
MTHFR C677T genotype — no.‡				
C/C	37	11	11	15
C/T	28	8	13	7
T/T	9	5	1	3

*Plus-minus values are means ±SD.

†The body-mass index is the weight in kilograms divided by the square of the height in meters.

‡Data for this variable were available for 74 subjects only.

§P=0.004 for the comparison with group A; other intergroup comparisons were not significant according to Duncan's multiple-range test for parametric values and Fisher's exact test for nonparametric values.

¶Values denote the number of subjects who consumed five or more servings per week.

||MTHFR denotes methylenetetrahydrofolate reductase.

take multivitamins (mean, 6.0 ± 3.6 ng per milliliter [13.6 ± 8.2 nmol per liter]) (unpublished data). Compliance with cereal-consumption instructions, estimated from returned breakfast-cereal packets, was similarly high for both the placebo and experimental cereals, ranging from 97.4 to 97.7 percent. The prevalence of the C677T methylenetetrahydrofolate reductase polymorphism, which influences sensitivity to the effects of folic acid on homocyst(e)ine levels,¹² was similar in the three groups.

Vitamin and Mineral Levels of the Cereals

The legend to Figure 1 lists the vitamins and minerals in the cereals. The average folic acid content of the group A experimental cereal (127 μ g of folic acid per 30 g of cereal) approximated the FDA-mandated daily additional intake from fortified cereal-grain products.⁵ The average folic acid content of the group B experimental cereal (499 μ g per 30 g of cereal) was about twice the currently accepted RDA of folic acid⁶ but similar to the composition of available "100 percent"-fortified cereals. This amount includes "overage," the amount of vitamins above the targeted level commonly added by the industry to account for the random variation associated with vitamin application and degradation during shelf-life. The folic acid content of the group C experimental cereal (665 μ g per 30 g of cereal) was selected to detect a potential threshold of effectiveness for homocyst(e)ine-lowering, previously calculated at about 400 to 500 μ g daily.¹⁶

Effects of Experimental Cereals on Plasma Homocyst(e)ine and Vitamin Levels

The basal plasma levels of homocyst(e)ine (Table 2) and folic acid (Table 3) did not differ significantly among the three groups before the placebo phase or the experimental phase.

The cereal containing an average of 127 μ g of folic acid per 30 g of cereal and the RDAs of other vitamins, including vitamins B₆ and B₁₂, reduced homocyst(e)ine by 3.7 percent ($P=0.24$). However, cereal fortified with an average of 499 or 665 μ g of folic acid per 30 g of cereal reduced homocyst(e)ine levels significantly, by 11.0 percent ($P<0.001$) and 14.0 percent ($P=0.001$), respectively. The homocyst(e)ine-lowering effects of the group A cereal were significantly less than those of the group B ($P=0.02$) or group C ($P=0.03$) cereal; the homocyst(e)ine-lowering effects of the cereals in groups B and C were not significantly different from each other ($P=0.53$). One outlier (with a homocyst(e)ine level of 66.8 μ mol per liter) accounted for higher basal levels in group C than in group A. However, exclusion of this outlier did not significantly affect the overall statistical results.

As compared with placebo, the cereal providing 127 μ g of folic acid daily increased plasma folic acid

levels by 30.8 percent ($P=0.045$), whereas the cereals providing 499 and 665 μ g of folic acid daily increased folic acid levels by 64.8 percent ($P<0.001$) and 105.7 percent ($P=0.001$), respectively. The experimental cereals provided the RDAs of vitamins B₆ and B₁₂ and increased the plasma levels of these vitamins, as compared with placebo (for B₆, 12.5 ng per milliliter [75 nmol per liter] with placebo vs. 15.0 ng per milliliter [90 nmol per liter] with the experimental cereals; $P<0.001$; and for B₁₂, 360 pg per milliliter [266 pmol per liter] with placebo vs. 408 pg per milliliter [301 pmol per liter] with the experimental cereals; $P<0.001$).

Homocyst(e)ine levels decreased linearly with increasing folic acid content of the cereal ($r=0.280$, $P=0.016$), whereas folic acid levels increased proportionately to the folic acid content of the cereal ($r=0.330$, $P=0.004$). The changes in plasma homocyst(e)ine levels were not significantly correlated with the changes in plasma vitamin B₁₂ levels ($r=-0.04$, $P=0.711$) or plasma pyridoxal 5'-phosphate levels ($r=-0.03$, $P=0.799$). In a stepwise regression analysis using the change in homocyst(e)ine levels as the dependent variable, the log of the change in folic acid levels was entered in step 1 ($r=-0.48$, $P<0.001$) and basal homocyst(e)ine levels in step 2 ($r=0.54$, $P<0.001$). These two variables accounted for 30 percent of the heterogeneity of the change in homocyst(e)ine levels.

DISCUSSION

An elevated plasma homocyst(e)ine level is a risk factor for arterial occlusive diseases that is present in about 13 percent of patients with coronary heart disease, 35 percent of patients with stroke, and 47 percent of patients with peripheral arterial occlusive disease.¹⁴⁻²⁰ Fortification of cereal-grain products with folic acid, as mandated by the FDA to prevent neural-tube defects, has the potential to reduce plasma homocyst(e)ine levels in patients with atherosclerosis. Although the clinical benefit of reducing homocyst(e)ine levels is unproved, it is hypothesized that the rate of atherosclerotic events may be reduced accordingly.¹⁶ In the present study, the homocyst(e)ine-lowering efficacy of folic acid-fortified cereals was tested in men and women with coronary artery disease.

The high rate of compliance with daily cereal consumption suggested that most subjects had changed their cereal consumption during the study, since only 49 percent had previously eaten five or more servings of breakfast cereal per week. This suggests that the general population may consume less fortified cereal and accrue less benefit from cereal fortified with folic acid than the subjects in this study.

The level of fortification proposed by the FDA is estimated to increase average daily folic acid intake by 70 to 120 μ g in adults older than 50 years.⁵ In

TABLE 2. EFFECTS OF BREAKFAST CEREALS ON PLASMA HOMOCYST(E)INE LEVELS.*

GROUP	MEAN FOLIC ACID CONTENT μg/30 g of cereal	HOMOCYST(E)INE LEVEL		MEAN CHANGE IN HOMOCYST(E)INE LEVEL (95% CI)†	P VALUE‡
		BASAL§	FOLLOW-UP		
		μmol/liter			
A (n=24)					
Placebo phase	10	9.9±3.1	10.1±3.7		
Experimental phase	127	10.0±3.0	9.5±2.5	-3.7 (-9.9 to 2.6)	0.24
B (n=25)					
Placebo phase	10	10.7±3.3	11.0±4.1		
Experimental phase	499	11.4±3.4	9.7±2.3	-11.0 (-15.5 to -6.6)	<0.001
C (n=26)					
Placebo phase	10	13.4±10.8	14.6±12.0		
Experimental phase	665	14.6±12.7	11.8±7.3	-14.0 (-21.2 to -6.2)	0.001

*Plus-minus values are means ±SD.

†P=0.02 for the comparison between group A and group B; P=0.03 for the comparison between group A and group C; P=0.53 for the comparison between group B and group C (by the Mann-Whitney U test). CI denotes confidence interval.

‡P values are for the difference from 0.0 of the mean percent change by the one-sample t-test.

§Analysis of variance was performed according to the Kruskal-Wallis test by ranks for all basal values; P=0.58.

TABLE 3. EFFECTS OF BREAKFAST CEREALS ON PLASMA FOLIC ACID LEVELS.*

GROUP	MEAN FOLIC ACID CONTENT μg/30 g of cereal	PLASMA FOLIC ACID LEVEL		MEAN CHANGE IN FOLIC ACID LEVEL (95% CI)†	P VALUE‡
		BASAL§	FOLLOW-UP		
		ng/ml¶			
A (n=24)					
Placebo phase	10	6.3±2.9	6.2±3.3		
Experimental phase	127	5.5±2.2	7.2±3.4	30.8 (0.68-60.97)	0.045
B (n=25)					
Placebo phase	10	8.9±5.1	6.9±2.9		
Experimental phase	499	7.4±3.7	10.3±3.1	64.8 (42.45-87.23)	<0.001
C (n=26)					
Placebo phase	10	7.3±3.2	6.9±3.2		
Experimental phase	665	6.3±2.8	12.5±5.0	105.7 (58.22-153.2)	<0.001

*Plus-minus values are means ±SD.

†P=0.012 for the comparison between group A and group B; P=0.001 for the comparison between group A and group C; P=0.171 for the comparison between group B and group C (by the Mann-Whitney U test). CI denotes confidence interval.

‡P values are for the difference from 0.0 of the mean percent change by the one-sample t-test.

§An analysis of variance was performed according to the Kruskal-Wallis test by ranks for all basal values; P=0.12.

¶To convert values for folic acid to nanomoles per liter, multiply by 2.266.

the present study, this amount of additional folic acid (127 μg daily), in combination with the RDAs of vitamins B₆ and B₁₂, was insufficient to reduce plasma homocyst(e)ine levels significantly and induced only moderate increases in folic acid levels. In contrast, levels of supplemental folic acid intake four

to five times as high reduced homocyst(e)ine levels 11.0 to 14.0 percent and increased folic acid levels 64.8 to 105.7 percent. Further dose-response studies using 100 to 400 μg of folic acid per day may be required to better delineate the interactions between low levels of cereal fortification with folic acid and

the regulation of homocyst(e)ine levels. The reduction of homocyst(e)ine levels with folic acid supplementation ranging from 0.2 to 15 mg per day without apparent toxicity has been reported.^{10-13,16,23,24}

The mechanisms responsible for the inverse relation between maternal folic acid intake and neural-tube defects are unknown. It is possible that this association may be related in part to adverse effects of homocyst(e)ine on fetal development. Several lines of evidence support this proposition. Elevated maternal homocyst(e)ine levels have been associated with pathologic outcomes of pregnancy,^{25,26} including an increased risk of neural-tube defects.²⁷ Moreover, among women whose children had neural-tube defects, homocyst(e)ine levels were higher at their first prenatal visit than among women whose children did not have neural-tube defects.²⁸ Currently available data are insufficient to distinguish the primary effects of low maternal folic acid intake from those of higher plasma homocyst(e)ine levels. However, if the beneficial effects of folic acid supplementation on neural-tube defects²⁻⁴ are related to reductions in homocyst(e)ine levels, it is possible that the FDA-proposed level of fortification may not adequately reduce the occurrence of neural-tube defects.

The similar homocyst(e)ine-lowering effects of the two breakfast cereals containing the highest levels of folic acid (499 μg and 665 μg per 30 g of cereal) support the concept that a near-maximal homocyst(e)ine-lowering efficacy of folic acid supplementation is often attained at a daily intake of about 400 μg .^{12,16} It seems likely that fortification of breakfast cereal with vitamin B₁₂ may conceivably obviate the potential risks of masking or exacerbating neurologic complications of vitamin B₁₂ deficiency, as discussed comprehensively by Tucker et al.²⁹ and Oakley.³⁰ Although all the experimental cereals contained the RDAs for several vitamins and minerals, including vitamins B₆ and B₁₂, several lines of evidence suggest that the effects on homocyst(e)ine levels were due to folic acid and not to the additional vitamins. First, cereal fortified with 127 μg of folic acid and with other vitamins and minerals did not lower homocyst(e)ine levels significantly. Second, linear and stepwise regression analyses demonstrated significant effects of folic acid but not of vitamins B₆ or B₁₂ on homocyst(e)ine lowering.

The heat-labile variant of methylenetetrahydrofolate reductase has reduced enzyme activity,³¹ and the responsible DNA mutation has been identified as a C-to-T mutation at nucleotide 677.^{21,32} Significant effects of the methylenetetrahydrofolate reductase polymorphism and folate status on plasma homocyst(e)ine levels have been thoroughly discussed by Jacques et al.³³ and Rozen.³⁴ Since persons homozygous for the C677T methylenetetrahydrofolate reductase polymorphism are more susceptible to the

homocyst(e)ine-lowering effects of folic acid supplementation,¹² our study population was stratified with regard to this polymorphism. The distributions of the methylenetetrahydrofolate reductase genotypes were similar in the three study groups and did not contribute to differences in homocyst(e)ine lowering among the three groups.

Conclusions

Fortification of breakfast cereals with folic acid levels approximating the increased daily intake specified by the FDA's enrichment policy for cereal-grain products did not have a significant effect on plasma homocyst(e)ine levels and had a moderate effect on folic acid levels in patients with coronary artery disease. In contrast, fortification at levels four to five times as high lowered plasma homocyst(e)ine levels 11.0 to 14.0 percent and increased plasma folic acid levels 64.8 to 105.7 percent. Boushey and colleagues predicted a substantial effect of folic acid fortification of food on cardiovascular disease through the lowering of homocyst(e)ine levels.¹⁶ They estimated that an increase in folic acid intake of 350 μg per day in men and 280 μg per day in women would potentially prevent 30,500 and 19,000 deaths from vascular causes per year, respectively.¹⁶

High homocyst(e)ine levels are strong predictors of death in patients with coronary artery disease,³⁵ but it remains to be established whether lowering homocyst(e)ine levels with vitamin therapy will decrease the risk of arterial occlusive diseases.^{36,37} Breakfast cereals are an important source of dietary folic acid,³⁸ and their intake is an important predictor of homocyst(e)ine levels.³⁹ The results of our investigation have expanded those findings by assessing the effects of different levels of folic acid fortification in breakfast cereals. Whether breakfast cereals fortified with folic acid will modify the incidence and outcome of atherosclerotic diseases needs to be established. Until these data are available, levels of fortification higher than the FDA recommends may be warranted.

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REFERENCES

1. Hibbard ED, Smithells RW. Folic acid metabolism and human embryopathy. *Lancet* 1965;1:1254.
2. Wagner WE, Levine B. Folic acid and neural tube defects. *Curr Concepts Nutr* 1993;8:1-12.
3. Czeizel AE, Dudás I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* 1992;327:1832-5.
4. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet* 1991;338:131-7.
5. Food standards: amendment of the standards of identity for enriched grain products to require addition of folic acid (21 CFR 136, 137, and 139). *Fed Regist* 1993;58(197):53305-5312.
6. Subcommittee on the Tenth Edition of the RDAs. Recommended dietary allowances. 10th ed. Washington, D.C.: National Academy Press, 1989.
7. Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. *MMWR Morb Mortal Wkly Rep* 1992;41(RR-14):1-7.
8. Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid (21 CFR 136, 137, and 139). *Fed Regist* 1996;61(44):8781-97.
9. Food labeling: health claims and label statements: folate and neural tube defects. *Fed Regist* 1993;58(197):53254-95.
10. Brattstrom L. Vitamins as homocysteine-lowering agents. *J Nutr* 1996;126:Suppl:1276S-1280S.
11. Landgren F, Israelsson B, Lindgren A, Hultberg B, Andersson A, Brattstrom L. Plasma homocysteine in acute myocardial infarction: homocysteine-lowering effect of folic acid. *J Intern Med* 1995;237:381-8.
12. Malinow MR, Nieto FJ, Kruger WD, et al. The effects of folic acid supplementation on plasma total homocysteine are modulated by multivitamin use and methylenetetrahydrofolate reductase genotypes. *Arterioscler Thromb Vasc Biol* 1997;17:1157-62.
13. Ubbink JB, Vermaak WJH, van der Merwe A, Becker PJ, Delpport R, Potgieter HC. Vitamin requirements for the treatment of hyperhomocysteinemia in humans. *J Nutr* 1994;124:1927-33.
14. Malinow MR. Hyperhomocyst(e)inemia: a common and easily reversible risk factor for occlusive atherosclerosis. *Circulation* 1990;81:2004-6.
15. Kang SS, Wong PWK, Malinow MR. Hyperhomocyst(e)inemia as a risk factor for occlusive vascular disease. *Annu Rev Nutr* 1992;12:279-98.
16. 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.
17. Mayer EL, Jacobsen DW, Robinson K. Homocysteine and coronary atherosclerosis. *J Am Coll Cardiol* 1996;27:517-27.
18. Duell PB, Malinow MR. Plasma homocyst(e)ine: an important risk factor for atherosclerotic vascular disease. *Curr Opin Lipidol* 1997;8:28-34.
19. Malinow MR, Kang SS, Taylor LM, et al. Prevalence of hyperhomocyst(e)inemia in patients with peripheral arterial occlusive disease. *Circulation* 1989;79:1180-8.
20. Malinow MR, Sexton G, Averbuch M, Grossman M, Wilson D, Upson B. Homocyst(e)inemia in daily practice: levels in coronary heart disease. *Coronary Artery Dis* 1990;1:215-20.
21. Goyette P, Sumner JS, Milos R, et al. Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. *Nat Genet* 1994;7:195-200.
22. Cunniff P, ed. Official methods of analysis of AOAC International. 16th ed. Vol. 1. Agricultural chemicals; contaminants; drugs. Gaithersburg, Md.: AOAC International, 1996.
23. Guttormsen AB, Ueland PM, Nesthus I, et al. Determinants and vitamin responsiveness of intermediate hyperhomocysteinemia (≥ 40 $\mu\text{mol/liter}$): the Hordaland Homocysteine Study. *J Clin Invest* 1996;98:2174-83.
24. Bostom AG, Shemin D, Lapane KL, et al. High dose-B-vitamin treatment of hyperhomocysteinemia in dialysis patients. *Kidney Int* 1996;49:147-52.
25. Wouters MG, Boers GH, Blom HJ, et al. Hyperhomocysteinemia: a risk factor in women with unexplained recurrent early pregnancy loss. *Fertil Steril* 1993;60:820-5.
26. Rajkovic A, Catalano PM, Malinow MR. Elevated homocyst(e)ine levels with preeclampsia. *Obstet Gynecol* 1997;90:168-71.
27. Steegers-Theunissen RP, Boers GH, Trijbels FJ, et al. Maternal hyperhomocysteinemia: a risk factor for neural-tube defects? *Metabolism* 1994;43:1475-80.
28. Mills JL, McPartlin JM, Kirke PN, et al. Homocysteine metabolism in pregnancies complicated by neural-tube defects. *Lancet* 1995;345:149-51.
29. Tucker KL, Mahnken B, Wilson PWF, Jacques P, Selhub J. Folic acid fortification of the food supply: potential benefits and risks for the elderly population. *JAMA* 1996;276:1879-85.
30. Oakley GP Jr. Let's increase folic acid fortification and include vitamin B-12. *Am J Clin Nutr* 1997;65:1889-90.
31. Kang SS, Wong PWK, Zhou J, et al. Thermolabile methylenetetrahydrofolate reductase in patients with coronary artery disease. *Metabolism* 1988;37:611-3.
32. 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.
33. Jacques PF, Bostom AG, Williams RR, et al. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation* 1996;93:7-9.
34. Rozen R. Molecular genetic aspects of hyperhomocysteinemia and its relation to folic acid. *Clin Invest Med* 1996;19:171-8.
35. Nygård O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med* 1997;337:230-6.
36. Malinow MR, Stampfer MJ. Role of plasma homocyst(e)ine in arterial occlusive diseases. *Clin Chem* 1994;40:857-8.
37. Stampfer MJ, Malinow MR. Can lowering homocysteine levels reduce cardiovascular risk? *N Engl J Med* 1995;332:328-9.
38. Albertson AM, Tobelmann RC, Marquart L. Folate consumption and the role of ready-to-eat cereal for American women aged 15 to 50 years. *Top Clin Nutr* 1997;12:58-68.
39. Shimakawa T, Nieto FJ, Malinow MR, Chambless LE, Schreiner PJ, Szklo M. Vitamin intake: a possible determinant of plasma homocyst(e)ine among middle-aged adults. *Ann Epidemiol* 1997;7:285-93.