

Breakfast cereal fortified with folic acid, vitamin B-6, and vitamin B-12 increases vitamin concentrations and reduces homocysteine concentrations: a randomized trial¹⁻³

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ABSTRACT

Background: High homocysteine and low B vitamin concentrations have been linked to the risk of vascular disease, stroke, and dementia and are relatively common in older adults.

Objective: We assessed the effect of breakfast cereal fortified with folic acid, vitamin B-6, and vitamin B-12 on vitamin and homocysteine status.

Design: A randomized, double-blind trial was conducted in 189 volunteers aged 50–85 y. The subjects had no history of hypertension, anemia, asthma, cancer, or cardiovascular or digestive disease and did not regularly consume multiple or B vitamin supplements or highly fortified breakfast cereal. Subjects were randomly assigned to consume 1 cup (0.24 L) breakfast cereal fortified with 440 μg folic acid, 1.8 mg vitamin B-6, and 4.8 μg vitamin B-12 or placebo cereal for 12 wk. Blood was drawn at 0, 2, 12, and 14 wk. Methionine-loading tests were conducted at baseline and week 14.

Results: Final baseline-adjusted plasma homocysteine concentrations were significantly lower and B vitamin concentrations were significantly higher in the treatment group than in the placebo group ($P < 0.001$). The percentage of subjects with plasma folate concentrations < 11 nmol/L decreased from 2% to 0%, with vitamin B-12 concentrations < 185 pmol/L from 9% to 3%, with vitamin B-6 concentrations < 20 nmol/L from 6% to 2%, and with homocysteine concentrations > 10.4 $\mu\text{mol/L}$ (women) or > 11.4 $\mu\text{mol/L}$ (men) from 6.4% to 1.6%. The percentage of control subjects with values beyond these cutoff points remained nearly constant or increased.

Conclusions: In this relatively healthy group of volunteers, consumption of 1 cup fortified breakfast cereal daily significantly increased B vitamin and decreased homocysteine concentrations, including post-methionine-load homocysteine concentrations. *Am J Clin Nutr* 2004;79:805–11.

KEY WORDS Fortification, folic acid, vitamin B-6, vitamin B-12, breakfast cereal, homocysteine, older adults

INTRODUCTION

Several studies have shown consistent and strong relations between homocysteine concentrations and heart disease, stroke, and other vascular outcomes (1–6). Most recently, there has been a growing interest in the association between homocysteine and cognitive decline (7–10). Homocysteine is an amino acid that is generated on the demethylation of methionine. It accumulates in the blood when there is an interruption in major metabolic path-

ways. The breakdown of homocysteine to cysteine requires a vitamin B-6–dependent enzyme. Remethylation to methionine requires a vitamin B-12–dependent enzyme, with folate as a substrate. The most common cause of homocysteine accumulation, therefore, is deficiency or low availability of folate, vitamin B-12, or vitamin B-6. The strong relations between these nutrients and homocysteine concentrations were clearly shown in the Framingham Heart Study cohort, in whom up to two-thirds of the cases of high homocysteine concentrations were associated with an inadequate folate, vitamin B-12, or vitamin B-6 status (11).

Evidence exists that low B vitamin intakes and high homocysteine concentrations are common problems among older adults in the United States. In the original cohort (aged 67–96 y) of the Framingham Heart Study, 29% had hyperhomocysteinemia—defined as a homocysteine concentration > 14 $\mu\text{mol/L}$. Since 1996, cereal grain products have been fortified with folic acid at 140 $\mu\text{g}/100$ g product. An analysis of data from the Framingham Offspring Study (children and spouses of the original cohort, aged 32–80 y) showed that folic acid fortification decreased the prevalence of high homocysteine concentrations (> 13 $\mu\text{mol/L}$) in nonsupplement users by almost one-half, from 18.7% before fortification to 9.8% after fortification (12). Those whose homocysteine concentrations did not respond to folic acid fortification may be responsive to supplementation with additional folic acid or to vitamins B-6 or B-12.

In one study, daily supplementation with 1, 10, and 0.4 mg folic acid, vitamin B-6, and vitamin B-12, respectively, was shown to normalize mildly elevated homocysteine concentrations within 6 wk (13). An alternative source of these 3 B vitamins is fortified breakfast cereal. We previously showed that the intake of fortified breakfast cereal was associated with higher plasma concentrations of folate and vitamin B-12 and with lower plasma homocysteine concentrations (14, 15). A study of patients with coronary artery disease found that daily intake of

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breakfast cereal fortified with 499 μg folic acid/serving increased plasma folate by 65% and decreased plasma homocysteine by 11% (16). In the current study we investigated, in a double-blind, randomized, controlled trial, the potential for breakfast cereal fortified with US recommended dietary allowances (RDAs) of folic acid, vitamin B-12, and vitamin B-6 to improve each of these vitamin concentrations and homocysteine status in a group of relatively healthy adults aged ≥ 50 y with no history of heart disease.

SUBJECTS AND METHODS

Subjects

We recruited volunteer subjects through advertisements in local newspapers, posters, radio, and mailing lists. Of 1705 persons who requested further information on the study, 622 applied for participation, and 603 were invited to participate. Of these 603 persons, 557 attended a screening session, at which they were asked about health conditions and medication and supplement use, were provided with details of the study requirements, and were asked to provide written informed consent. At that visit, 113 of these persons decided not to continue in the study, primarily because of perceived time constraints but also because they were either unwilling or unable to conform with the study procedures. Another 205 persons were disqualified at screening for the following reasons: an exclusionary health condition (eg, digestive disease, cardiovascular disease, uncontrolled hypertension, anemia, asthma, or cancer; $n = 77$), regular use of multivitamin or B vitamin supplements ($n = 69$), use of medications that might interfere with B vitamin metabolism ($n = 16$), regular use of highly fortified breakfast cereal or other dietary supplement fortified with B vitamins ($n = 32$), current participation in another study ($n = 5$), inadequate health insurance ($n = 4$), poor venous access ($n = 1$), or a language barrier ($n = 1$). The remaining subjects, therefore, were generally healthy adults (aged ≥ 50 y) who were not taking vitamin supplements.

Of the 239 subjects enrolled in the study, 24 were lost during the protocol for the following reasons: time constraints or loss of interest ($n = 11$), refusal to follow study procedures ($n = 6$), development of a medical condition ($n = 4$), or supplement use ($n = 3$). Two hundred fifteen subjects (93 men and 122 women) completed the protocol, of whom 196 were non-Hispanic white, 12 were African American, 4 were Asian American, and 3 were of another ethnicity. Of the subjects who participated, 26 had missing data for one or more variables. Complete data for most analyses were available for 93 subjects in the treatment group and for 96 subjects in the control group; 10 of these subjects refused at least one methionine-load test, which left 89 subjects in the treatment group and 90 subjects in the control group for the analysis. All procedures were reviewed and approved by the Tufts New England Medical Center Human Investigations Review Committee.

Methods

The study was a double-blind, randomized, controlled trial. The enrolled subjects were randomly assigned to consume breakfast cereal fortified with the RDAs of folic acid, vitamin B-6, and vitamin B-12 (400 μg , 2 mg, and 6 μg , respectively) per 1-cup (0.24 L) serving or an identical cereal without the addition of these vitamins. Analysis of the ready-to-eat cereal after fortification showed that the actual content was 440 μg folic acid, 1.8

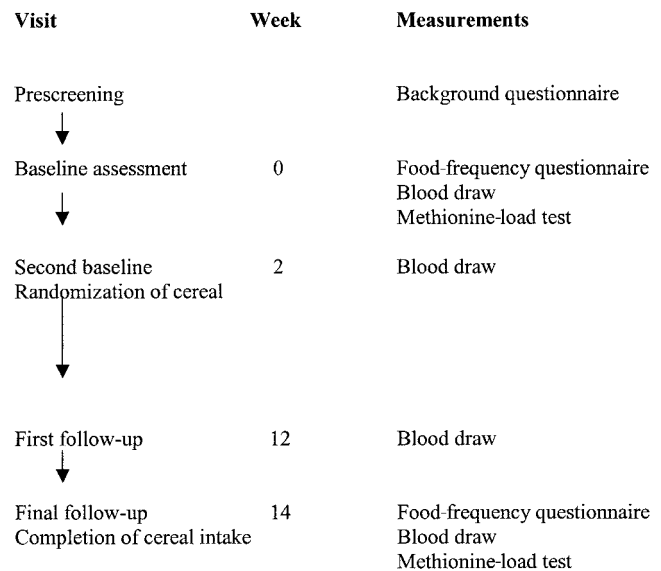


FIGURE 1. Study protocol.

mg vitamin B-6, and 4.8 μg vitamin B-12 per serving. Both cereals contained 100 kcal, 24 g carbohydrate, 1 g dietary fiber, 0.35 mg thiamine, 0.34 mg riboflavin, and 4.0 mg niacin per serving. The study statistician (GED) randomly assigned the subjects to 1 of the 2 groups. All staff members that interacted with the subjects were blind to the group assignments.

Qualified subjects were asked to make 4 visits to the study center in a fasting (12 h) state (Figure 1). At the first visit, the subjects completed a validated 126-item semiquantitative food-frequency questionnaire (17) and had blood drawn for the measurement of plasma folate, vitamin B-12, and vitamin B-6 [as pyridoxyl-*P* (PLP)]. A methionine-load test was administered. Subjects were asked to drink a preparation that contained 100 mg L-methionine/kg body wt suspended in apple juice. Blood was drawn exactly 2 h later to measure the elevation of postload homocysteine concentrations. After 2 wk, the subjects returned to have their blood drawn for the second time. At that visit, each subject was randomly assigned to a study group, provided with the appropriate cereal, and instructed to consume one serving of cereal with milk per day. The subjects were reminded to not consume other breakfast cereals and to not use vitamin supplements during the study; no other changes to the diet were required. The subjects returned to have their blood drawn for the third time, 10 wk after the second visit. The subjects continued to consume the cereal for another 2 wk and then made their final visit to the study center. At the final visit, the subjects completed a second food-frequency questionnaire that asked them to report their diet over the previous 3 mo, had blood drawn for the fourth time, and underwent a final methionine-load test.

Subjects were asked to bring any remaining cereal and empty cereal cartons to the last visit, as a check on compliance. Ninety-five percent of the subjects returned their empty boxes; 82% had consumed $\geq 95\%$ of the allotted cereal, 94% had consumed $\geq 90\%$ of the allotted cereal, and only 2% ($n = 4$) had consumed $< 85\%$ of the allotted cereal.

Vitamin and homocysteine analysis

The cereal prepared with added vitamins was analyzed at the headquarters of the Kellogg Company (Battle Creek, MI) to

verify content. Vitamin B-12 was measured by comparing the growth response of the sample in the bacteria *Lactobacillus delbrueckii* with the growth response of a vitamin B-12 standard. Folic acid was extracted in a basic solution of methanol and water, cleaned on an anion-exchange column, and quantified by HPLC with postcolumn derivation to produce an oxidized folic acid derivative, which is detected by fluorescence. Vitamin B-6 was extracted in an acidic solution of methanol and water and quantified by reversed-phase HPLC with fluorescence detection.

Plasma samples were stored at -80°C until completion of the study and were analyzed together at the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University for all subjects with complete data. Total homocysteine in plasma was measured with the use of an adaptation of the method described by Araki and Sako (18). The CV for this assay in our laboratory is 4.0%. PLP was determined enzymatically with the use of tyrosine decarboxylase based on the principles described by Shin-Buehring et al (19). The CV for this assay in our laboratory is 5.0%. Plasma folate and vitamin B-12 concentrations were determined by radioassay with a commercially available kit from Bio-Rad (Hercules, CA). The CVs for these assays in our laboratory are 4.7% for vitamin B-12 and 4.3% for folate.

Statistical analysis

Subject characteristics, reported nutrient intakes, and plasma concentrations of folate, vitamin B-12, PLP, and homocysteine at baseline were compared across treatment groups with a two-sample *t* test for continuous variables or with a chi-square test for categorical variables. We averaged the plasma measures from the first 2 visits to obtain stable measures of baseline status for folate, vitamin B-12, PLP, and homocysteine. Similarly, the last 2 blood measures were averaged for each analysis as the posttreatment measures. Post-methionine-load homocysteine concentrations were measured only at baseline and at the end of the study. Differences in change in plasma measures across groups were assessed with repeated-measures analysis of variance. To assess the generalizability of the full response, we tested the interactions between the plasma vitamin responses to the vitamin-fortified cereal and 1) baseline plasma vitamin values (values above compared with values below the median) and 2) age (> 65 y compared with ≤ 65 y). When the interactions were significant ($P < 0.01$), we repeated the analyses separately in those with baseline plasma vitamin values above or below the median and in the 2 identified age groups. All analyses were performed in SAS for WINDOWS (version 8.02; SAS Institute Inc, Cary, NC).

RESULTS

There were no significant baseline differences across treatment group by age, sex, ethnicity, BMI, marital status, education level, smoking status, alcohol use, or medication use (Table 1; all $P > 0.05$). In both groups, the average age of the subjects was 65 ± 9 y, there were slightly more women than men, and $> 90\%$ of the subjects were non-Hispanic white. More than one-half of the subjects in each group were currently married, and nearly 50% had college degrees. The average BMI (in kg/m^2) in both groups of subjects was in the overweight category, 26–27. Few of these subjects (3–6%) reported being current smokers, and their average alcohol intake was low (< 6 g to ≈ 0.5 drinks/d). Although the subjects had been screened for serious illness and for use of

TABLE 1
Subject characteristics¹

	Treatment group (n = 93)	Control group (n = 96)
Age (y)	65.4 \pm 9.3 ²	65.1 \pm 8.8
Male (%)	41.0	48.0
BMI (kg/m^2)	26.2 \pm 3.9	26.9 \pm 5.0
College graduate (%)	44.9	42.7
Married (%)	53.6	62.5
Ethnicity (%)		
Non-Hispanic white	94.0	90.0
African American	4.0	5.0
Other	2.0	5.0
Smoking (%)	3.2	6.4
Alcohol intake (g/d)	4.0 \pm 6.5	5.5 \pm 8.1
Use of medications (%)		
Heart disease	1.6	1.1
Hypertension	12.7	10.6
Estrogen	12.9	12.5

¹ Variables were compared across groups by *t* test for continuous and chi-square for categorical variables. There were no significant differences between groups ($P > 0.05$).

² $\bar{x} \pm \text{SD}$ (all such values).

medications known to affect B vitamin metabolism, 3 subjects in each group reported the use of heart medication, 24 subjects in the treatment group and 20 subjects in the control group reported the use of antihypertensive medication, and 12 subjects in each group reported the use of estrogen.

Intakes of folate, vitamin B-6, and vitamin B-12 did not differ significantly between groups at baseline (Table 2; $P > 0.05$). Average daily intakes of folate and vitamin B-6 in the treatment and control groups were 365 and 375 dietary folate equivalents and 2.4 and 2.1 mg/d, respectively; intakes of both vitamins approximated the RDA (20). Average vitamin B-12 intakes were 6.4 and 7.0 $\mu\text{g}/\text{d}$, respectively; these intakes are greater than the current RDA (20). With test cereal replacement, intakes of these B vitamins decreased somewhat in the control group (Table 2). Although subjects that were consuming highly fortified cereals at baseline were excluded from participation, many of the enrolled subjects were consuming partially fortified cereals at baseline. In

TABLE 2
Average daily vitamin intake¹

	Treatment group (n = 93)	Control group (n = 96)
Folate (DFE) ²		
Baseline	375 \pm 17	365 \pm 18
14 wk ³	745 \pm 14	292 \pm 17
Vitamin B-12 (μg)		
Baseline	6.4 \pm 0.5	7.0 \pm 0.7
14 wk ³	9.8 \pm 0.3	6.1 \pm 0.5
Vitamin B-6 (mg)		
Baseline	2.3 \pm 0.1	2.1 \pm 0.1
14 wk ³	3.7 \pm 0.1	1.8 \pm 0.1

¹ All values are $\bar{x} \pm \text{SEM}$. There were no significant differences in intake across groups at baseline by *t* test comparison ($P > 0.05$).

² DFE, dietary folate equivalents, with naturally occurring food folate: 1 $\mu\text{g} = 1$ DFE for fortified foods and 1 $\mu\text{g} = 1.7$ DFE (20).

³ Intakes were based on the analysis of the food-frequency questionnaire for all foods other than the cereal and the B vitamins from the test cereal.

TABLE 3
Plasma vitamin and homocysteine concentrations¹

Variable	Treatment group (n = 93)	Control group (n = 96)
Folate (nmol/L)		
Baseline, 0–2 wk	24.7 ± 0.7	24.7 ± 0.7
12–14 wk ²	32.2 ± 0.7	22.4 ± 0.7
Vitamin B-12 (pmol/L)		
Baseline, 0–2 wk	296 ± 10	292 ± 10
12–14 wk	354 ± 13	290 ± 10
Homocysteine (μmol/L)		
Baseline, 0–2 wk	7.9 ± 0.2	7.8 ± 0.2
12–14 wk	7.5 ± 0.2	8.2 ± 0.2
Vitamin B-6 ³ (nmol/L)		
Baseline, 0–2 wk	51.8 ± 4.9	45.9 ± 2.6
12–14 wk	82.3 ± 5.5	42.1 ± 2.4
Post-methionine-load homocysteine (μmol/L) ⁴		
Baseline	22.7 ± 0.8	22.9 ± 0.8
14 wk	21.3 ± 0.7	24.0 ± 0.8

¹ All values are $\bar{x} \pm \text{SEM}$. The difference in change across the treatment and control groups was significant for all measures, $P = 0.0001$ (repeated-measures ANOVA).

² Average of measures taken at 0 and 2 wk (baseline) and at 12 and 14 wk for all variables except post-methionine-load homocysteine, which were taken at baseline and 14 wk only.

³ As pyridoxal-5'-phosphate.

⁴ $n = 89$ for the treatment groups and 90 for the control group.

the control group, these partially fortified cereals were replaced with cereals that had not been fortified with folate, vitamin B-6, and vitamin B-12, which resulted in a slight decrease in intake of these vitamins in the control group. None of the average baseline plasma measures differed significantly between groups ($P > 0.05$) and were within currently defined normal ranges.

Despite the fact that these subjects had been consuming diets fortified with folic acid for several years, there was a significant increase in plasma folate in the treatment group (from 25 to 32 nmol/L) but a slight, nonsignificant decrease (from 25 to 22 nmol/L) in the control group (**Table 3**). The difference in change across groups was significant ($P < 0.0001$). Vitamin B-12 concentrations also increased significantly in the treatment group, from ≈ 296 to 354 pmol/L. In comparison, vitamin B-12 concentrations did not change significantly in the control group (range: 290–292 pmol/L). Although the average baseline fasting homocysteine concentrations were relatively low in this healthy group of older adults, they decreased significantly (from 7.9 to 7.5 μmol/L; $P < 0.0001$) in the treatment group but increased (from 7.8 to 8.2 μmol/L; NS) in the control group (Table 3).

The largest treatment response was evident for vitamin B-6, on the basis of PLP concentrations. PLP concentrations increased almost 60% in the treatment group, from 52 to 82 nmol/L, relative to a decrease (from 46 to 42 nmol/L) in the control group ($P < 0.0001$). Although vitamin B-6 has also been shown to be associated with fasting homocysteine concentrations, it has a greater effect on the metabolic pathway that converts homocysteine to cysteine and, therefore, is better reflected in the transient concentrations of homocysteine that increase and decrease after a meal. For this reason, in addition to fasting homocysteine concentrations, we also examined homocysteine concentrations 2 h after a methionine load (Table 3). As with the other measures,

TABLE 4
Percentage of subjects with vitamin values below or above selected cutoffs

	Treatment group (n = 93)	Control group (n = 96)
	%	
Folate < 11 nmol/L		
Baseline	3.2	0
12–14 wk	0	0
Vitamin B-12 < 185 pmol/L		
Baseline	9.7	11.5
12–14 wk	3.3	11.5 ¹
Vitamin B-6 < 20 nmol/L ²		
Baseline	5.4	7.3
12–14 wk	1.1	9.4 ¹
Homocysteine > 10.4 (women) or 11.4 (men) μmol/L ³		
Baseline	12.9	10.4
12–14 wk	3.2	7.3

¹ Significantly different from the treatment group, $P < 0.05$ (chi-square test).

² As pyridoxal-5'-phosphate.

³ See reference 21.

there was a significant decrease in this measure of homocysteine in the treatment group relative to the control group ($P < 0.0001$). In the treatment group, post-methionine-load homocysteine concentrations decreased from 22.7 to 21.3 μmol/L, whereas an increase in the comparison group was observed. No significant interactions were observed between treatment and age (> 65 y compared with ≤ 65 y) for any of the plasma vitamin measures or between treatment and baseline values of PLP or vitamin B-12 above or below the median. However, the subjects with baseline plasma folate values below the median of 25 nmol/L had a greater response to the treatment than did those with initially higher plasma folate concentrations (increases of 12.0 and 2.2 nmol/L, respectively). Similarly, the subjects with baseline plasma homocysteine values greater than the median of 7.8 μmol/L had an average decrease in homocysteine of 0.9 μmol/L after treatment compared with a decrease of 0.4 μmol/L after treatment in subjects with baseline plasma homocysteine values below the median. When tested separately, the results remained significant ($P < 0.05$) for each of these subgroups.

Few of the healthy volunteers had vitamin concentrations that were below clinical cutoffs for deficiency, but improvements were seen in the treatment group (**Table 4**). At baseline, no significant differences in the proportions of values below or above the specified cutoffs were observed (chi-square test: $P > 0.05$); 3.2% of the treatment group and none of the control group had plasma folate concentrations < 11 nmol/L. After the intervention, none of the subjects had low folate concentrations on the basis of this criterion. With the use of a concentration < 185 pmol/L as a cutoff for vitamin B-12, 9.7% of the treatment group and 11.5% of the control group had low plasma concentrations at baseline. This proportion fell to 3.2% in the treatment group but remained constant in the control group after 12 wk of intervention. These values differed significantly after the intervention ($P > 0.05$). Similarly, the percentage of treatment subjects with plasma PLP < 20 nmol/L decreased from 5.4% to 1.1%, whereas the percentage of control subjects with low plasma PLP rose from 7.3% to 9.4%. Finally, the 13% of treatment subjects with fasting homocysteine concentrations > 10.4 μmol/L (women) or 11.4

$\mu\text{mol/L}$ (men) at baseline were reduced to 3.2%, relative to a smaller percentage decrease, from 10.4% to 7.3% in control subjects. Cutoffs for homocysteine were based on the 95th percentile of concentrations from a nationally representative sample of adults aged 20–39 y (21).

DISCUSSION

In this double-blind, randomized, controlled trial of fortified cereal use, we found that one serving per day of breakfast cereal fortified with approximately the US RDAs of folic acid, vitamin B-6, and vitamin B-12 led to significant improvements in the plasma concentrations of each of these vitamins and in both fasting and post-methionine-load homocysteine concentrations. To our knowledge, this is the first study to show such a response in postload homocysteine concentrations. These results were seen in a relatively healthy group of adults aged 50–85 y, who had been consuming a diet fortified with folic acid for the past several years.

These findings are important for several reasons. First, the evidence that elevated homocysteine concentrations contribute to the risk of heart disease and stroke is strong (1–5), and there is increasing evidence of its contribution to cognitive decline (9, 10, 22). In addition, there is also evidence that low normal concentrations of these 3 B vitamins may contribute directly to the impaired health of older adults. Low folate status has been associated directly with acute coronary events (23), with brain atrophy (24), and with memory impairment (25). Although the recent fortification of the food supply has improved folate status tremendously, our results show that there remains room for improvement in some persons.

We and others showed previously that low vitamin B-12 concentrations are common among older adults (15, 26, 27), and there is evidence that this may lead to a variety of cognitive and neurologic consequences, even at levels formerly considered borderline normal and in patients without anemia or macrocytosis (28, 29). Studies of vitamin B-12 status among older adults have consistently identified large segments of the population as either deficient or “low normal” despite apparently adequate dietary intakes of vitamin B-12. Because of the need for several steps in the separation of vitamin B-12 from food protein and preparation for absorption, which require an acidic environment in the stomach, many older adults have difficulty absorbing vitamin B-12. This problem, which has been estimated to affect up to 40% of the elderly, is associated with impaired absorption of protein-bound vitamin B-12 (30). Bioavailability studies have shown that free cobalamin, as found in vitamin supplements and in the form added to fortified breakfast cereals, is better absorbed than is vitamin B-12 bound to proteins in food (31). For this reason, the recently released dietary reference intakes by the Food and Nutrition Board of the Institute of Medicine include the recommendation that most of the vitamin B-12 intake in persons older than 50 y should come from supplements or fortified foods (32). Our finding that vitamin B-12 concentrations improved significantly after consumption of the fortified cereal supports our previous observation that cereal use is associated with vitamin B-12 status (15). Nonusers of supplements who consume cereal > 4 times/wk are 50% less likely to have low vitamin B-12 concentrations than are those who do not consume cereal or supplements.


Vitamin B-6 is important to immune function (33, 34), and low plasma concentrations are associated with heart disease, independent of homocysteine concentrations (35–37). A study by Friso et al (38) found that plasma vitamin B-6 is associated with markers of inflammation. Associations have also been identified between plasma vitamin B-6 and depression (39, 40). Although vitamin B-6 is known to affect the clearance of postload homocysteine (41), it has not been shown that improvements in plasma PLP via the consumption of foods fortified with vitamin B-6 can lead to significantly lower post-methionine-load homocysteine concentrations. Although much less is known about the health effects of sustained postload homocysteine concentrations, there is evidence that it has negative effects on platelet aggregation and endothelial function (42, 43).

Since the introduction of folic acid fortification of cereal grain products, the associations between vitamin intakes and fasting homocysteine concentrations have shifted from being primarily dependent on folate status to being more dependent on the status of vitamin B-12 and vitamin B-6. Although the prevalence of a low folate status was greatly reduced by fortification, only $\approx 50\%$ of those with high homocysteine concentrations ($> 13 \mu\text{mol/L}$) fell below that cutoff after fortification (from 19% of the adult population in the Framingham Offspring Study before, to $\approx 10\%$ after fortification) (12). The inclusion of vitamins B-12 and B-6 in fortified breakfast cereal is, therefore, likely to have an additional effect on the remaining high homocysteine concentrations. We found that the combination of folic acid, vitamin B-6, and vitamin B-12 together reduced the prevalence of high plasma homocysteine in this group of healthy subjects from 13% to 3%.

The subjects in this study were purposefully selected to represent relatively healthy older adults and were not prescreened for low vitamin status. These results should, therefore, be generalizable to older adults without known cardiovascular disease or other major health conditions who do not regularly use vitamin supplements. However, in addition to not using vitamin supplements for 2 mo before the start of the study, the subjects were selected only if they were not regularly consuming highly fortified (but not partially fortified) breakfast cereals. It is likely that the reduction in plasma vitamin concentrations in the control group was partially due to the replacement of the partially fortified cereals they may have been consuming before the study began with the nonfortified study cereals.

Because volunteers for intervention studies are generally more knowledgeable and motivated by health issues than is the general population, it is likely that the general population has a greater frequency of poor vitamin status and could benefit from regular cereal consumption to an even greater extent than our study subjects. Because many older adults choose not to take vitamin supplements, the consumption of fortified cereal may be an attractive means of obtaining required amounts of folic acid, vitamin B-6, and vitamin B-12.

Together, these results showed that a daily serving of breakfast cereal fortified at approximately the current RDAs of folic acid, vitamin B-6, and vitamin B-12 can make an important contribution to improving the status of these vitamins as well as to lowering both fasting and postload homocysteine concentrations. This is important because, although vitamin supplement use appears to be increasing, data from the third National Health and Nutrition Examination Survey (1988–1994) indicate that 59% of non-Hispanic white men and 45% of non-Hispanic white women reported using no vitamin supplements. These proportions were

greater for non-Hispanic blacks (70% and 58% for men and women, respectively) and Mexican Americans (64% and 55%, for men and women, respectively) (44). It is therefore possible for large segments of the older adult population to benefit from the consumption of fortified breakfast cereal. Because each of these vitamins has been shown to have an important role in protecting vascular, neurologic, and immune function and in contributing to a reduction in homocysteine concentrations, the consumption of fortified breakfast cereals may offer significant health benefits to older adults. 

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