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# The effect of fortified breakfast cereal on plasma homocyst(e)ine concentrations in healthy older men already consuming a folate fortified diet

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## Abstract

High plasma homocysteine (hcy) concentrations may be an independent risk factor for cardiovascular disease. Folic acid supplementation reduces plasma hcy, however no studies used fortified foods and nutrition education to promote a sustained diet change. The effects of nutrition education and fortified cereal consumption on plasma hcy were studied in 41 men  $\geq$  58 years old. Subjects randomized into the experimental group ( $n=21$ ) received super-fortified ready-to-eat cereal (400  $\mu\text{g}$  folic acid) for 8 weeks, and nutrition education based on the Social Cognitive Theory. Control group subjects ( $n=20$ ) received general nutrition education only. Folate intake at baseline was similar to recommended levels ( $436 \pm 204 \mu\text{g}/\text{day}$ ). Experimental subjects increased folic acid intake, and serum folate was significantly higher at follow up. Plasma hcy did not differ significantly over time between groups. Subjects with moderate hcy concentrations who are already eating fortified foods regularly may not benefit from consuming folate above recommended levels. © 2003 Elsevier Inc. All rights reserved.

*Keywords:* Homocysteine; Folate; Aging; Men

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## 1. Introduction

Considerable research has shown that a high homocysteine (hcy) level in the blood is an independent risk factor for coronary artery disease (CAD) [1–5]. A study involving the

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elderly Framingham Study population demonstrated a twofold increase in all-cause cardiovascular disease mortality among those in the highest quartile of hcy compared with those in the lowest hcy quartile [6]. Homocysteine appears to be similar to cholesterol in that there is, as yet, no known threshold for the atherogenicity of this amino acid, so that the higher the plasma hcy the greater the risk, even within the normal reference range [3,7]. Folic acid supplementation has been shown to reduce levels of plasma hcy [8–12].

In 1998, the Food and Drug Administration (FDA) began requiring that all producers of fortified cereal grain products add folic acid (140  $\mu\text{g}/100\text{ g}$ ) to their products [13]. The FDA allows more folic acid to be added to ready-to-eat (RTE) cereals. Most cereals provide 25% of the RDA or 100  $\mu\text{g}$  of the RDA; however, highly fortified cereals provide 400  $\mu\text{g}$  of folic acid per serving [14]. The level of fortification of grain products recommended by the FDA was estimated to increase daily folic acid intake by 70 to 120  $\mu\text{g}$  in adults over 50 years old [15]. Prior to fortification most Americans consumed less than 400  $\mu\text{g}$  folate/day, the current RDA [16] and the amount that is recommended to reduce the risk of heart disease [17,18]. Since fortification, intake of grain products enriched with folic acid has been associated with an improvement in folate status [15,19–21]. Three studies, conducted before fortification of most grain products, demonstrated that folic acid fortified breakfast cereals produced a modest reduction in hcy over a relatively short time [15,21,22]. However, no studies have combined nutrition education with cereal consumption to determine if older adults will maintain recommended diet changes.

The objective for this research was to investigate the effects of nutrition education and a high folate diet on homocysteine in healthy older men. In light of the fortification of grain products just prior to this study and the interest in using food as a medium for delivery of important vitamins and minerals we sought to determine if daily consumption of a fortified food (at recommended levels) in addition to normal diet would have an impact on hcy levels in this group of men. Men in the intervention group participated in nutrition education built upon the social cognitive (social learning) theory [23] and an anthropological theory of food patterning [24,25], and consumed highly fortified RTE cereals.

## 2. Subjects and Methods

### 2.1. Participants

Participants in this study were 41 Caucasian men ( $\geq 58$  years of age) with no known serious medical condition who were not taking supplements that contained folic acid or medications known to affect hcy (e.g. methotrexate, anticonvulsant agents, bile acid sequestrants). In order to participate, potential subjects who were taking a folic acid containing supplement completed a 12 week washout phase in which they discontinued taking any folic acid containing supplements. All subjects gave informed consent and the study was approved by the Institutional Review Boards at Oklahoma State University and Oklahoma Medical Research Foundation. Subjects were enrolled starting in October 1999 and data collection ended in June 2000.

## 2.2. Experimental design

At recruitment, all subjects provided demographic, medication and supplement data, completed a 24 hr diet recall, and started four 1-day food records. All subjects were given complete instructions and food record supplies (measuring cups, spoons, and beanbag standard measures) to increase the accuracy of their record [26]. All subjects then completed a semiquantitative food frequency questionnaire (FFQ) [27]. At baseline, approximately 1 week after the first visit, subjects returned the food record and were randomized into either the experimental (n=21) or control (n=20) group. Blood samples were taken at baseline, on weeks 4 and 8, and three months post-intervention (week 20). A trained interviewer obtained unannounced 24-hour dietary recalls by telephone during weeks 3, 7, and 19, and subjects completed 5-day food records after completion of the study (after week 8), and 3 months post intervention (week 20). Food records and recalls were analyzed using the dietary software program Food Processor (version 7.4, ESHA Research, 1999).

## 2.3. Nutritional education

Subjects in the experimental group were instructed on ways to increase folate in their diet using behavioral constructs from the social cognitive theory including how to choose high folate foods from a restaurant menu and how to interpret the amount of folate from a food label. Subjects were also given a self-monitoring folder that contained an individualized “folate in foods” counter and a more extensive general list of folate containing foods. The counter consisted of a list of the amount of folate provided by each subject’s core and secondary core foods [25] that was developed from their initial food frequency questionnaire. They also received a self-monitoring log to record the amount and type of folate-containing food consumed. The dietary information was reinforced at the 4 week visit, and additional encouragement and information was provided at the 8 and 20 week visits using the 4 week and the post 8 week 5-day diet record analyses.

Participants in the control group were instructed on a healthy diet using the Food Guide Pyramid [28]. A handout regarding increasing fruit, vegetable, and grain consumption was also provided [29]. They were not provided with cereal or a self monitoring folder. The nutrition education for both groups was provided by one Registered Dietitian (TSH).

## 2.4. Fortified cereal

The fortified RTE cereals provided for participants in the experimental group contained 400µg of folic acid plus 100% Daily Value (DV) [30] for other vitamins and minerals including vitamins B-6 (2 mg) and B-12 (6 mg) (Table 1). Subjects were asked to consume the cereal daily for 8 weeks in addition to their usual diet. Subjects were given 30 packets of pre-measured cereal at both the baseline and 4 week visits. Each packet contained one serving by weight as indicated on the Nutrition Facts label of either: General Mills Total Raisin Bran™; General Mills Whole Grain Total™; General Mills Multigrain Cheerios Plus™; or Kellogg’s Smart Start™. To increase compliance, subjects were allowed to choose their cereal. At both the 4 and 8 week visits, the empty and remaining packets of cereal were returned and counted for an assessment of compliance.

As an alternative to eating the cereal, the subjects were taught how to choose an equivalent amount of folate using a point system. Subjects could choose foods that equaled 10 points

Table 1  
Composition of super-fortified cereals

Nutrient	Whole Grain Total <sup>TM</sup>		Total Raisin Bran <sup>TM</sup>		Multi-Grain Cheerios Plus <sup>TM</sup>		Smart Start <sup>TM</sup>	
	Amount	% DV	Amount	% DV	Amount	% DV	Amount	% DV
Serving Size	30 g		55 g		30 g		50 g	
Vitamin A	500 IU	10	500 IU	10	500 IU	10	750 IU	15
Vitamin C	15 mg	25	0 mg	0	15 mg	25	15 mg	25
Calcium	250 mg	25	250 mg	25	100 mg	10	0 mg	0
Iron	15 mg	100	5 mg	100	15 mg	100	15 mg	100
Vitamin D	40 IU	10	40 IU	10	40 IU	10	40 IU	10
Vitamin E	30 IU	100	30 IU	100	30 IU	100	30 IU	100
Thiamin	1.5 mg	100	1.5 mg	100	1.5 mg	100	1.5 mg	100
Riboflavin	1.7 mg	100	1.7 mg	100	1.7 mg	100	1.7 mg	100
Niacin	20 mg	100	20 mg	100	20 mg	100	20 mg	100
Vitamin B-6	2 mg	100	2 mg	100	2 mg	100	2 mg	100
Folic Acid	400 $\mu$ g	100	400 $\mu$ g	100	400 $\mu$ g	100	400 $\mu$ g	100
Vitamin B-12	6 $\mu$ g	100	6 $\mu$ g	100	6 $\mu$ g	100	6 $\mu$ g	100
Pantothenic Acid	10 mg	100	10 mg	100	10 mg	100	10 mg	100
Phosphorus	80 mg	8	100 mg	10	100 mg	10	100 mg	10
Magnesium	24 mg	6	40 mg	10	24 mg	6	32 mg	8
Zinc	15 mg	100	15 mg	100	15 mg	100	15 mg	100
Copper	0.08 mg	4	0.16 mg	8	0.04 mg	2	0.08 mg	4

% DV is Percent Daily Value

(each point was equivalent to 40  $\mu$ g folic acid) from the individualized list of folate-containing foods developed from their initial FFQ. If the subjects chose to eat foods with naturally occurring folate (i.e. legumes, fruits, vegetables) instead of the fortified cereal they were required to consume 40% more folate because of the difference in bioavailability of naturally occurring folate compared to synthetic folic acid in the fortified cereal [31]. Foods chosen and corresponding point values were recorded using self-monitoring logs. The logs were reviewed to evaluate compliance at the week 4 and 8 visits.

### 2.5. Biochemical analyses

At each visit, blood samples (after 10-12 h fast) were collected in Vacutainer tubes; one containing EDTA for plasma collection and one untreated for serum samples. The plasma was processed immediately at 3000 rpm in a refrigerated centrifuge at 4° C, and frozen in aliquots at -20° C. The processed plasma was used for analysis of plasma total homocysteine by high performance liquid chromatography and fluorescence detection using minor modifications of the methods of Vester and Rasmussen [32] and Ubbink et al. [33]. The intra assay CV of the internal standards was 1.96%. Additional plasma aliquots were used to analyze total and HDL cholesterol, the ferric reducing ability of plasma (FRAP), and creatinine using Roche reagents in a COBAS Roche FARA II chemistry system. FRAP provides an estimate of "antioxidant power" [34]. Creatinine was measured because it has a positive relationship with hcy [35]. The intra assay CVs of the internal controls for total cholesterol, HDL cholesterol, FRAP, and creatinine were 1.4%, 2.3%, 1.7% and 1.6%, respectively.

Serum tubes were stored on ice for 30 minutes to allow clot formation before being

centrifuged and frozen at  $-20^{\circ}$  C. Serum was analyzed for serum folate, vitamin B-12, and erythrocyte (RBC) folate by radioimmunoassay (Cobra II, Auto-Gamma Counter; Packard Instrument Co.) using the Dualcount Solid Phase No Boil Assay (Diagnostic Products Corporation 1999). The inter assay CVs for anemia controls were 6.4% for folate, and 9.6% for B-12. All samples were thawed only once for analysis.

### 2.6. Statistical analyses

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) 10.0 for Windows [36]. Sample size was estimated using data from O’Keefe et al. [37] in Hall’s [38] method. One sample *t*-tests were used to determine if subjects met the estimated average requirement (EAR) and RDA [16] for folate, vitamins B-12 and B-6. Analysis of variance (ANOVA) was used to compare the groups on dietary folate intake. Repeated measures analyses of variance were used to determine if there were changes in dietary folate, hcy, serum folate, serum vitamin B-12, erythrocyte (RBC) folate, and FRAP over time and/or by group. Bonferroni post hoc tests were performed to adjust for multiple comparisons. The baseline measure was used as a covariate if there was a significant difference in baseline measures between the groups. Linear regression was used to determine if there were associations between hcy concentration and biochemical and dietary variables. The selection of variables for regression was based on those shown in previous studies to be significant determinants of hcy concentration. Variables chosen were folate intake, coffee consumption, smoking status [39], total cholesterol, HDL cholesterol, serum vitamin B-12, serum folate [5], vitamin B-6 intake [1], RBC folate [40] and serum creatinine [5,8,35]. Independent sample *t*-tests were used to compare biochemical measures for differences between groups at the follow-up visit (3 months post-intervention). Logarithmic transformations of the variables were performed as needed to improve normality. However, untransformed values were used to construct tables and graphs of summary statistics. Data are reported as mean  $\pm$  standard deviation. Differences were considered statistically significant at the  $p \leq 0.05$  level [41].

## 3. Results

Characteristics for the two groups of subjects were similar at entry. There were no significant differences between groups for any of the demographic characteristics (Table 2). Reported dietary intakes are shown in Table 3. Men’s estimated intake of energy ranged from approximately 1850 to 2200 kcal/day throughout the study period, and total fat intake resembled that of the “typical” American diet (approximately 33-35% of calories). There were no significant differences in energy or fat intake between groups or over time.

Analysis of the diet records for both groups indicated that estimated mean intake of folate at baseline was  $436 \pm 204 \mu\text{g/day}$  which was not significantly different from the RDA of 400  $\mu\text{g}$  daily at any of the measured times. However, folate intake estimated by the diet record was significantly higher than the estimated average requirement (EAR) at baseline, post intervention (after week 8), and 20 weeks ( $p=0.001$ , 0.008, 0.002, respectively). Even so, 35.5% of the men did not meet the EAR of 320  $\mu\text{g/day}$  and an additional 16% did not meet

Table 2  
 Characteristics of subjects at entry, according to study groups

Characteristics	Control Group (n=20)	Experimental Group (n=21)
Age-yr	66 ± 6*	65 ± 5
BMI <sup>a</sup>	28 ± 4	28 ± 4
Employment status-no. (%)		
Full time	8 (40)	9 (43)
Part time	5 (25)	3 (14)
Retired	7 (35)	9 (43)
Education level-no. (%)		
High school	4 (20)	3 (14)
Other training-no college	4 (20)	—
Some college	3 (15)	—
Bachelors degree	2 (10)	7 (33)
Masters degree	4 (20)	4 (19)
Doctorate degree	3 (15)	7 (33)
Following a special diet- no. (%) <sup>b</sup>		
No	17 (85)	17 (81)
Smoke -no. (%) <sup>b</sup>		
No	16 (80)	17 (81)
Cups of coffee/day- no. (%)		
0-2	9 (45)	13 (62)
>2	11 (55)	8 (38)

\* Plus-minus values are means ± SD

<sup>a</sup> The body mass index is the weight in kilograms divided by the square of the height in meters.

<sup>b</sup> Data on this variable was available for 40 subjects only

the RDA. Analysis of 24-hr. recalls revealed that folate intake at week 3 was significantly higher than the RDA ( $p < 0.001$ ), and folate intake at weeks 3, 7, and 19 was significantly higher than the EAR ( $p < 0.001, 0.001, 0.024$ , respectively).

Repeated measures analyses revealed a significant ( $p = 0.001$ ) time by group interaction for dietary folate. Folate intake in the experimental group was significantly higher than controls at week 3. Intake at week 3 in the experimental group was significantly higher than at baseline, weeks 8, 19, and 20, and intake at week 7 was higher than at baseline and week 8 ( $p < 0.05$ ). Folate intake in the control group did not change significantly over time (Fig. 1).

Mean intake for vitamins B-12 and B-6 exceeded the RDA ( $2.4 \mu\text{g}$  and  $1.7 \text{mg}$ , respectively) [16] for this age group throughout the study. There was a significant time by group interaction ( $p = 0.013$ ) for intake of vitamin B-12. Week 3 intake was significantly higher than at baseline for the experimental group ( $p < 0.05$ ). There was a significant interaction ( $p = 0.000$ ) for dietary intake of vitamin B-6. (Table 3). Vitamin B-6 intake in the experimental group was significantly higher than controls at week 3. Intake at week 3 in the experimental group was significantly higher than at baseline, weeks 8, 19, and 20, and intake at week 7 was higher than at baseline and week 8 ( $p < 0.05$ ). Compliance with cereal-consumption instructions, estimated from returned breakfast cereal packets plus logs of consumption of other folate-containing foods, was  $95.6 \pm 6.1\%$  for the first month, and  $93.3 \pm 6.3\%$  the second month.

Table 3  
Dietary intake of subjects by treatment group at six time points based on 5-day diet records and 24-hr recalls<sup>†</sup>

	Energy (kcal)	Folate $\mu\text{g}$	Vitamin B-12 ( $\mu\text{g}$ )	Vitamin B-6 (mg)	Total Fat (% energy)
<b>Control Group:</b>					
Baseline <sup>a</sup>	2079 $\pm$ 428	404 $\pm$ 157	8.05 $\pm$ 6.31	2.23 $\pm$ 0.85	33 $\pm$ 8
week 3 <sup>b</sup>	1899 $\pm$ 530	341 $\pm$ 156 <sup>†</sup>	5.28 $\pm$ 2.55	1.77 $\pm$ 0.87 <sup>†</sup>	34 $\pm$ 12
week 7 <sup>b</sup>	2150 $\pm$ 470	388 $\pm$ 196	33.1 $\pm$ 83.2	2.19 $\pm$ 1.18	36 $\pm$ 9
Post 8-week <sup>*a</sup>	1944 $\pm$ 427	393 $\pm$ 48	6.22 $\pm$ 4.02	2.16 $\pm$ 1.10	33 $\pm$ 9
week 19 <sup>b</sup>	1991 $\pm$ 423	341 $\pm$ 166	5.82 $\pm$ 3.90	2.05 $\pm$ 0.98	33 $\pm$ 10
week 20 <sup>a</sup>	1924 $\pm$ 401	361 $\pm$ 165	5.91 $\pm$ 3.13	1.99 $\pm$ 0.96	33 $\pm$ 10
<b>Intervention Group:</b>					
Baseline <sup>a</sup>	1979 $\pm$ 478	393 $\pm$ 140 <sup>z</sup>	4.98 $\pm$ 1.95 <sup>x</sup>	2.04 $\pm$ 0.67 <sup>z</sup>	33 $\pm$ 9
week 3 <sup>b</sup>	1922 $\pm$ 600	684 $\pm$ 195 <sup>†x</sup>	11.3 $\pm$ 7.90 <sup>y</sup>	3.44 $\pm$ 1.02 <sup>†x</sup>	32 $\pm$ 10
week 7 <sup>b</sup>	1879 $\pm$ 606	576 $\pm$ 198 <sup>x,y</sup>	8.29 $\pm$ 3.04	4.24 $\pm$ 4.64 <sup>x,y</sup>	33 $\pm$ 9
Post 8-week <sup>*a</sup>	1855 $\pm$ 399	397 $\pm$ 112 <sup>z</sup>	5.41 $\pm$ 2.32	2.06 $\pm$ 0.53 <sup>z</sup>	32 $\pm$ 8
week 19 <sup>b</sup>	1868 $\pm$ 446	456 $\pm$ 251 <sup>y,z</sup>	7.42 $\pm$ 4.64	2.44 $\pm$ 1.05 <sup>y,z</sup>	36 $\pm$ 11
week 20 <sup>*</sup>	1919 $\pm$ 434	483 $\pm$ 194 <sup>y,z,z</sup>	7.22 $\pm$ 3.31	2.49 $\pm$ 0.84 <sup>y,z</sup>	35 $\pm$ 7

\* 8-week records were collected after the intervention ended

‡ Plus-minus values are means  $\pm$  SD.

<sup>a,b</sup> Superscript a indicates diet record, b indicates 24-h recall.

<sup>†</sup> Different superscripts in a column indicate significant differences between groups ( $p < 0.05$ )

<sup>x,y,z</sup> Different superscripts in a column indicate significant differences between times ( $p < 0.05$ )

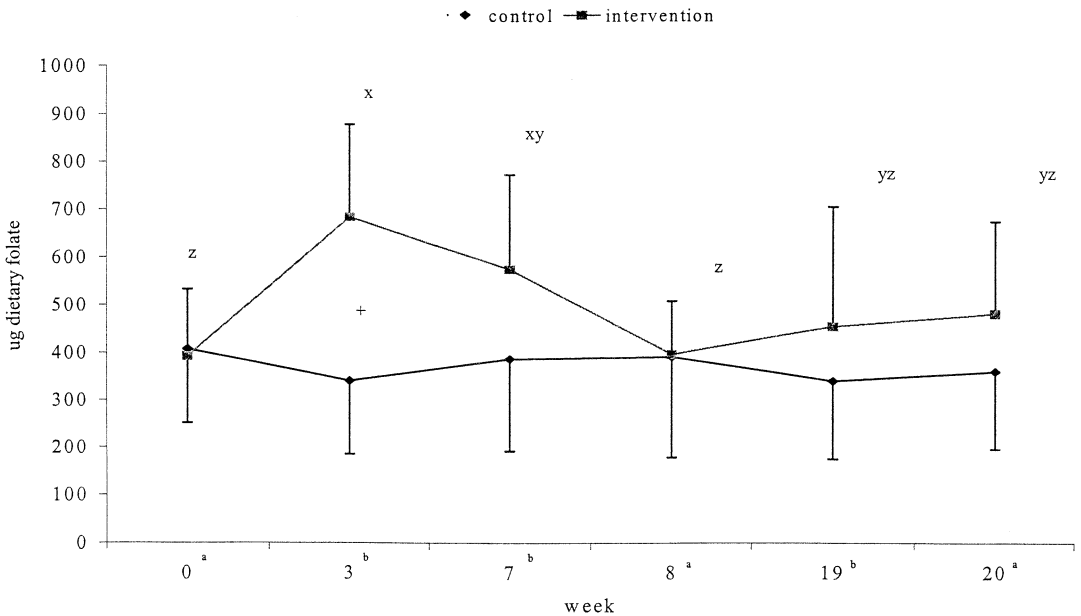


Fig. 1. Mean ( $\pm$ SD) folate intake from food estimated by diet records<sup>a</sup> and 24-hr recalls.<sup>b</sup> Points with a superscript indicate means that are different between groups (+) or over time (x,y,z) for the interaction using repeated measures ANOVA.

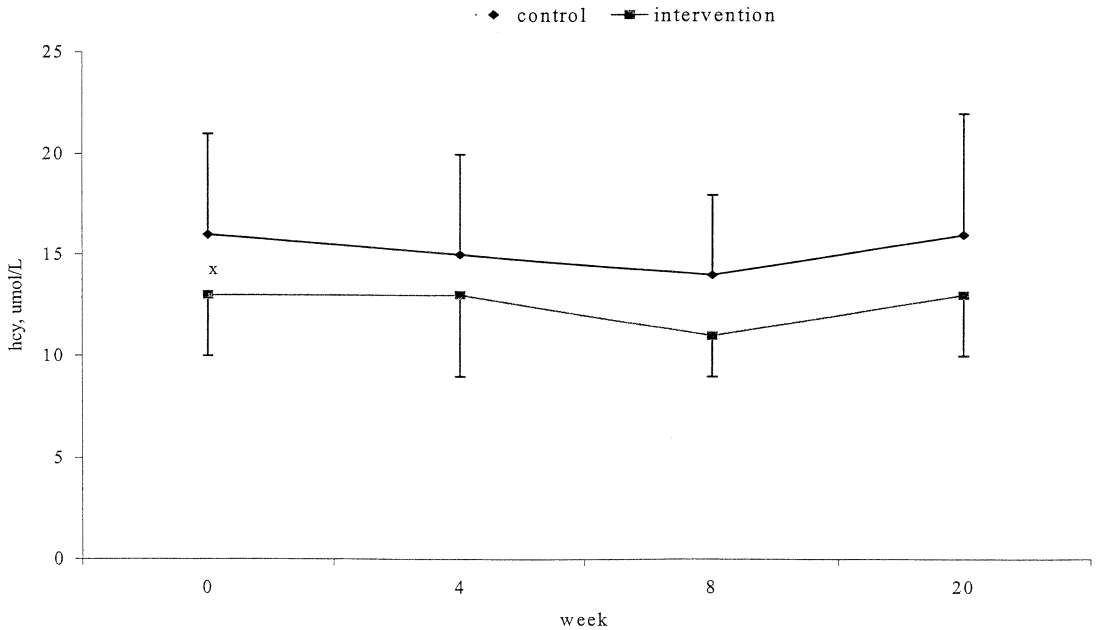


Fig. 2. Mean ( $\pm$ SD) plasma homocysteine (hcy) over 4 time periods and between groups. Points with superscripts (x) indicate means that are different between the groups using *t*-test.

The mean hcy concentration of the entire study group was  $14.5 \pm 4.6$   $\mu\text{mol/L}$  (range: 8.3-28.5  $\mu\text{mol/L}$ ) at baseline. All participants had concentrations of serum and RBC folate, and serum vitamin B-12 that were within or above their respective reference ranges (3-17 ng/mL, 175-700 ng/mL, 200-950 pg/mL) (Diagnostic Products Corporation 1999) [42]. Mean creatinine was within the reference range (0.6-1.5 mg/dL), however 6 subjects in the control group and 6 subjects in the experimental group had creatinine values slightly above the reference range (Diagnostic Products Corporation 1999) [42].

Plasma hcy was significantly different between the groups at baseline ( $p = 0.008$ ) therefore, baseline hcy was used as a covariate in repeated measures analysis of variance. At baseline the control group had more subjects with elevated ( $\geq 14$   $\mu\text{mol/L}$ ) hcy concentrations ( $n = 13$ , 65%) than the experimental group ( $n = 7$ , 33.3%). Once baseline hcy was controlled, there was no significant difference in hcy between groups over time (Fig. 2).

Creatinine and serum vitamin B-12 were significantly correlated with hcy; 23% of the variance in hcy was explained by these variables at baseline ( $p = 0.009$ ) (Beta= 0.393  $p = 0.019$ , Beta=  $-0.300$   $p = 0.048$ , respectively) and 39% was explained at the end of the intervention period ( $p < 0.001$ ) (Beta= 0.452  $p = 0.001$ , Beta=  $-0.430$   $p = 0.002$ , respectively). Creatinine was positively correlated, and serum vitamin B-12 was negatively correlated with hcy. The other variables in the model were not significantly related to hcy (age, smoking status, cups of coffee per day, total cholesterol, HDL, LDL, FRAP, dietary and serum folate, and dietary vitamin B-12, data not shown).

Serum folate concentrations showed a significant time by group interaction ( $p = 0.040$ ). Serum folate in the experimental group was significantly higher at week 20 than at baseline

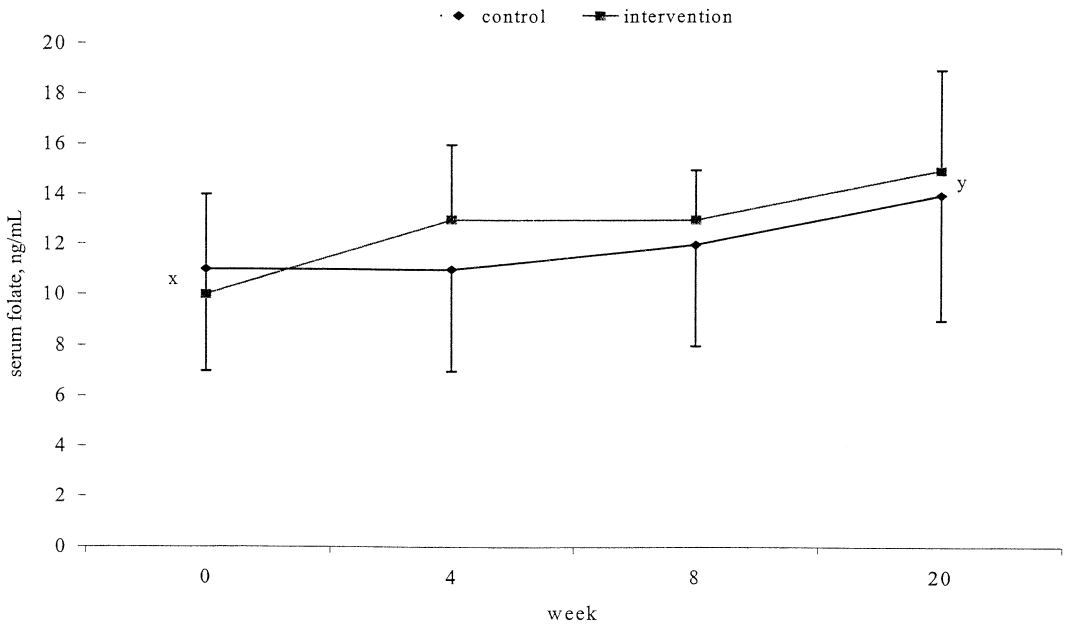


Fig. 3. Mean ( $\pm$ SD) serum folate over 4 time periods and between groups. Points with different superscripts (x, y) indicate means that are different across time for the interaction using repeated measures ANOVA.

( $p < 0.05$ ) (Fig. 3). However, serum folate was not significantly different over time in the control group. There was no significant difference in RBC folate between groups over time, although RBC folate for both groups was significantly lower at baseline, weeks 4 and 8 than

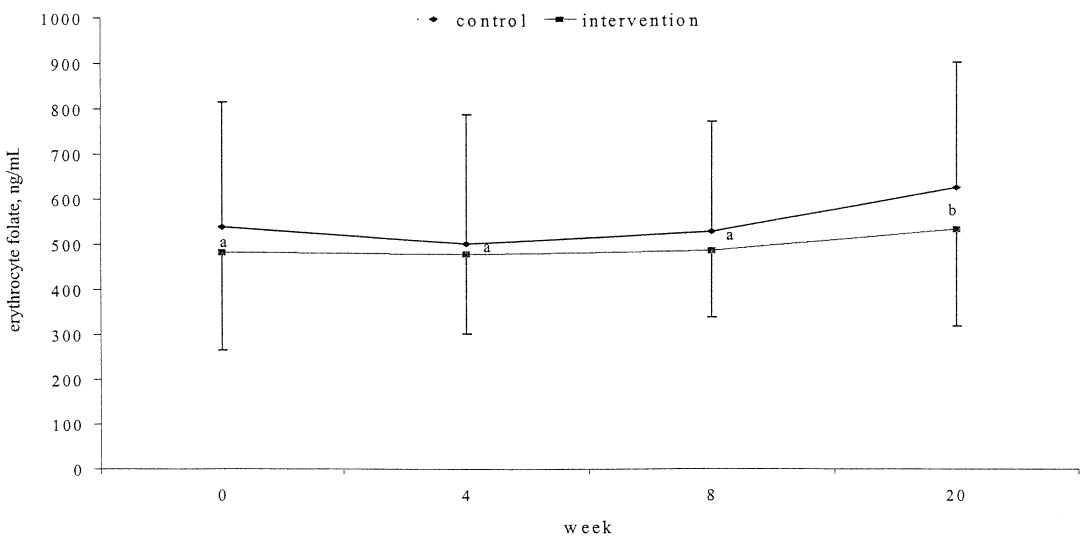


Fig. 4. Mean ( $\pm$ SD) erythrocyte folate for experimental and control groups. Points with different superscripts (a,b) indicate means that are different across time using repeated measures ANOVA.

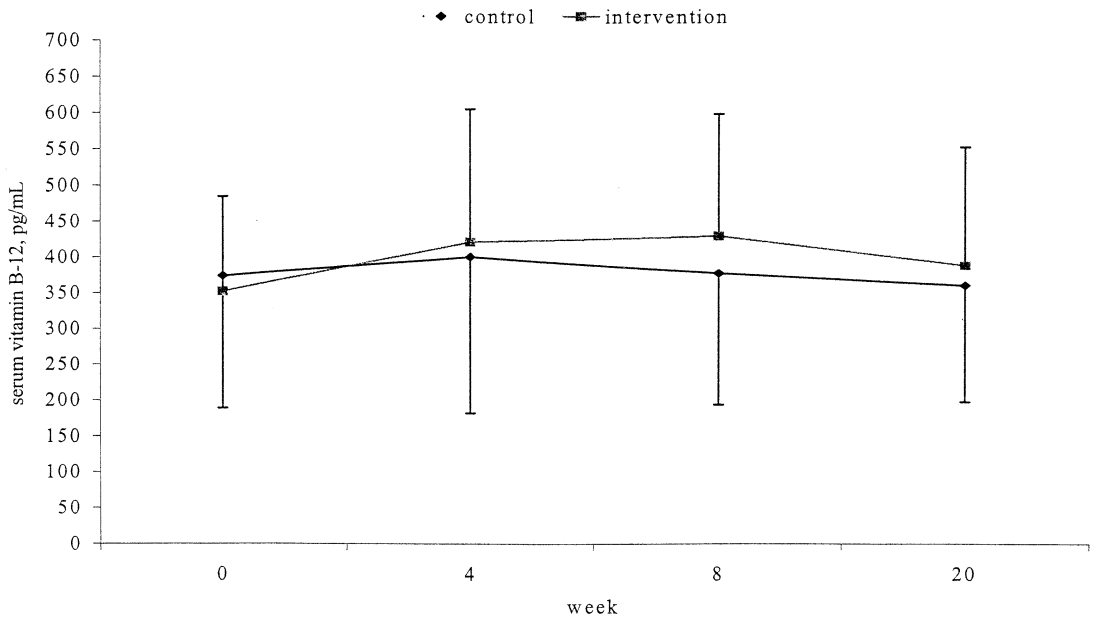


Fig. 5. Mean ( $\pm$ SD) serum vitamin B-12 for experimental and control groups.

at week 20 ( $p = 0.046$ ) (Fig. 4). Time by group interaction was significant for serum vitamin B-12 ( $p = 0.024$ ) due to differences in pattern of intake (Fig. 5). Neither FRAP nor creatinine concentrations (data not shown) were significantly different over time or between groups.

#### 4. Discussion

It has been known for some time that daily supplementation with 400  $\mu\text{g}$  folic acid reduces hcy concentrations in healthy individuals [9–11,22,43,44]. Several studies conducted before widespread folic acid fortification reported similar findings. For example, Malinow et al. [15] observed reductions in hcy of 11% and 14%, respectively, in male and female CHD patients consuming cereals fortified with 499 and 665  $\mu\text{g}$  folic acid/serving. Schorah et al. [21] observed a 10% reduction in hcy concentrations after 24 weeks when healthy individuals with a low intake of fortified foods or supplemental folic acid consumed cereals fortified with 200  $\mu\text{g}$  folic acid. Riddell et al. [22] observed a 24% reduction in hcy concentrations in subjects with  $\text{hcy} \geq 9 \mu\text{mol/L}$  consuming an additional 298  $\mu\text{g/d}$  of folic acid in fortified breakfast cereals for 12 weeks.

The impact of fortification on the food supply was evident in a recent report from the Framingham Offspring Cohort [49]. Researchers found that fortification significantly improved folate status. In another study, Bostom et al. [50] found that 131 CAD patients exposed to cereal grain flour products fortified with folic acid who received 2.5 mg/d folic acid supplements for 12 weeks experienced only very modest reductions (1  $\mu\text{mol/L}$ ) in mean fasting plasma hcy levels. Rydlewicz et al. [51] concluded that a total daily intake of 926

$\mu\text{g}/\text{d}$  (from food and supplement) would be required to ensure that 95% of the elderly population would be without cardiovascular risk from folate deficiency. In the present study, the hcy lowering efficacy of fortified cereals with 400  $\mu\text{g}$  folic acid, 6  $\mu\text{g}$  vitamin B-12, and 2 mg vitamin B-6 was tested in healthy older men for 8 weeks.

For this group of men the change in hcy between groups over time was not significant. Schorah et al. [21] found that folic acid did not decrease hcy for those already in the lowest tertile for hcy, and the higher the initial hcy, the greater the folate effect. Similarly, a meta-analysis of hcy lowering trials showed that a proportionate and absolute reduction in blood hcy produced by folic acid supplements was greatest in subjects with higher pretreatment hcy and lower pretreatment blood folate concentrations [44]. Few subjects in the experimental group had elevated pretreatment concentrations of hcy or low serum folate concentrations. It is conceivable that the moderate baseline concentrations of hcy and the normal pretreatment serum folate concentrations in the experimental group in our study may have reduced the hcy-lowering potential of the dietary treatment.

Schorah et al. [21] saw an increase in serum folate at weeks 4, 8 and 24 in subjects eating fortified cereal. Similarly, Malinow et al. [15] saw an increase in plasma folate proportionately with folic acid content of cereal. In our study, the experimental group's serum folate increased between baseline and week 4 and remained higher during the second half of the intervention (week 8) and the post intervention period (week 20). Red blood cell folate in this study, which typically takes approximately 3-4 months to respond to dietary change [21], was significantly lower at baseline, week 4 and week 8 than at week 20 for both groups. Red blood cell folate did not change until 24 weeks in a similar study [21]. The observed increase for vitamin B-12 in this study's experimental group occurred during weeks 4 and 8 and remained higher at week 20. Schorah et al. [21] found no significant change in vitamin B-12 over a 24-week period, even though one of the groups was eating cereal fortified with vitamin B-12 in addition to folic acid and other vitamins.

Mean dietary folate intake at baseline (before cereal consumption) for those in the experimental group was already  $393 \pm 140$  and did not significantly increase after the intervention ended. However, mean folate intake in the experimental group increased at weeks 3 ( $684 \pm 195 \mu\text{g}$ ) and 7 ( $576 \pm 198 \mu\text{g}$ ), while the control group's intake did not change ( $341 \pm 156 \mu\text{g}$ ,  $388 \pm 196 \mu\text{g}$ , respectively). The high rate of compliance with daily cereal consumption suggested that most subjects in the experimental group were eating the provided cereal throughout the study, however prior to the study 76% ( $n=16$ ) of those subjects were already regular cereal eaters. The cereal that was provided may have been a replacement of the cereals they were already eating, or for some the provided cereal was eaten in addition to other cereals. According to diet records, 70% ( $n=14$ ) of the control group ate cereal prior to the study, 65% ( $n=13$ ) ate cereal on at least 2 of 4 measured occasions, although only 3 of the cereal eaters were eating super-fortified cereals.

Most participants in the control group already ate recommended amounts of folate [ $404 \pm 157$ ] at baseline and continued to meet at least the EAR of 320  $\mu\text{g}/\text{d}$  [ $361 \pm 165$ ] at the end of the study period (20 weeks). The high intake of folate in the control group may explain why there were no differences in RBC folate and hcy between the groups over the study period. Perhaps subjects from both groups in this study had already reached a plateau of folate intake prior to the study. Fortification of grain products prior to the start of this study

may have already had some effect [45,19]. Several groups have suggested a near maximal hcy lowering effect of supplemental folic acid at intakes of approximately 400  $\mu\text{g}/\text{d}$  [3,10,15,37,44].

The homocysteine-lowering ability of folic acid fortified cereals in subjects who were already consuming 400  $\mu\text{g}$  folate per day was not apparent in this study. The subjects in this study were mostly highly educated older men and the consistently high levels of folate intake throughout the study period indicated that this group was already paying attention to their diets, and may have already been aware of the health benefits of consuming adequate folate. Many of these men were already regular cereal eaters who apparently replaced their regular cereal with the super-fortified cereal only during the 8 week period. After the intervention stopped, the targeted education did not seem to increase these men's folate intake above the current recommendation of 400  $\mu\text{g}/\text{day}$ . It is possible that subjects with mildly elevated hcy concentrations who are already eating fortified foods regularly may require only simple dietary advice to continue to maintain plasma hcy concentrations in the normal range.

Future research to test the effects of folic acid fortified cereals should be conducted in a group of men who are not typical cereal eaters. In addition, blinding the subjects by using a placebo cereal may also prove to have an effect by discouraging those not asked to change their diet (control group) from doing so. In similar studies using folic acid fortified cereals the subjects were receiving either a placebo cereal or a cereal with a different amount of fortification [15,21,22]. Malinow et al. [15] asked participants to replace previously eaten breakfast cereals with the placebo and experimental cereals that were provided. In this study, participants in the control group did not receive a placebo cereal, and some were aware that subjects in the other group were receiving cereal. Therefore, participants in the control group not already eating cereal may have decided to eat similar foods on their own causing a similar increase in biochemical measures of folate.

Including the spouse in the visits may have increased the effectiveness of the targeted nutrition education. Spouses, especially wives, monitor and attempt to control their partner's health behaviors [46], and having them present might have influenced the continued intake of high folate containing foods beyond the intervention period. Finally, recruiting subjects who would benefit the most, those who have elevated hcy concentrations and/or low serum folate concentrations, would likely show the most improvement.

Breakfast cereals are an important source of dietary folic acid [47], and their intake is an important predictor of hcy concentrations [48]. Our investigation has expanded those findings by indicating that fortification of grain products may have already had an effect on this group of men even before intake of super-fortified breakfast cereals. Further intervention trials to find the lowest effective dose and best combination of B vitamins that lowers hcy concentrations is needed. More importantly, whether breakfast cereals fortified with folic acid will modify the incidence and outcome of atherosclerotic disease still needs to be established.

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