

Dietary strategies for lowering homocysteine concentrations¹⁻³

Lynnette J Riddell, Alexandra Chisholm, Sheila Williams, and Jim I Mann

ABSTRACT

Background: Elevated plasma total homocysteine (tHcy) concentrations are associated with increased risk of vascular disease, and there is a strong inverse association between dietary and blood folate and blood tHcy concentrations. Increased folate consumption may lower the risk of tHcy-mediated cardiovascular disease.

Objectives: The objective was to determine the most appropriate means of increasing dietary folate to reduce plasma tHcy.

Design: Sixty-five free-living subjects aged 36–71 y with tHcy concentrations ≥ 9 $\mu\text{mol/L}$ participated in a randomized, controlled trial to compare 3 approaches for increasing dietary folate to ≈ 600 $\mu\text{g/d}$: folic acid supplementation, consumption of folic acid–fortified breakfast cereals, and increased consumption of folate-rich foods.

Results: An intake of 437 μg folic acid/d from supplements resulted in a 27-nmol/L increase in serum folate and a 21% reduction in tHcy, relative to the change in a control group. In subjects who consumed folic acid–fortified breakfast cereal, folate intake increased by an average of 298 μg , serum folate increased by 21 nmol/L, and tHcy concentrations decreased by 24%. Increased intakes of folate-rich foods resulted in a 418- μg increase in dietary folate, a 7-nmol/L increase in serum folate, and a 9% reduction in tHcy concentrations. The decrease in tHcy was negatively correlated ($r = -0.66$) with the increase in serum folate.

Conclusions: Daily consumption of folic acid–fortified breakfast cereals and the use of folic acid supplements appear to be the most effective means of reducing tHcy concentrations. The reduction in tHcy was significantly negatively correlated with the increase in serum folate, which may be a useful marker for measuring dietary change. *Am J Clin Nutr* 2000;71:1448–54.

KEY WORDS Homocysteine, dietary folate, folic acid, fortified breakfast cereals, serum folate, free-living subjects, cardiovascular disease

INTRODUCTION

Elevated total homocysteine (tHcy) concentrations have been confirmed as a risk factor for ischemic heart disease (IHD) and other vascular disorders (1). There are no clinical trial data to confirm that a reduction in tHcy concentrations will reduce clinical vascular events or mortality but there is widespread agreement that an elevated tHcy concentration is an independent risk factor, as powerful as other classic risk factors such as hypertension and hypercholesterolemia (2). The mechanism by which an elevated tHcy concentration is involved in the pathogenesis

of vascular disease has not been established with certainty, but experimental evidence suggests several possibilities: endothelial cell toxicity (3), proliferation of smooth muscle cells (4), and thrombus formation (5).

Cross-sectional studies have shown a strong inverse association between dietary and blood folate concentrations and blood tHcy concentrations (1, 6). Increased concentrations of blood folate lead to an increase in 5-methylenetetrahydrofolate, which in turn increases the rate at which homocysteine is remethylated to methionine (7). Folic acid given as a dietary supplement in tablet form has been shown to reduce tHcy concentrations, with doses between 0.5 and 5.0 mg producing a reduction of $\approx 25\%$. There is no convincing evidence that doses at the higher end of this range produce a more marked effect than do doses at the lower end of this range (8). Two studies showed that folic acid–fortified breakfast cereals produce a modest reduction in tHcy over a relatively short time (9, 10). We report here a comparison of 3 approaches to reduce tHcy concentrations by increasing dietary folate to ≈ 600 $\mu\text{g/d}$: consumption of folic acid supplements, consumption of folic acid–fortified breakfast cereals, and an increased consumption of folate-rich foods. An intake of 600 μg folate/d was selected because the results of folate supplementation studies suggest little or no additional lowering of tHcy with greater intakes, and from a practical point of view it is difficult to increase the consumption of folate-rich foods in free-living individuals to an extent that will result in higher dietary intakes.

SUBJECTS AND METHODS

Subjects

Sixty-five subjects (25 women and 40 men aged 36–71 y) with tHcy concentrations ≥ 9 $\mu\text{mol/L}$ were recruited from 431 volunteers responding to advertisements in a local newspaper and in

¹From the Departments of Human Nutrition and Preventive and Social Medicine, the University of Otago, Dunedin, New Zealand.

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³Address reprint requests to JI Mann, Department of Human Nutrition, University of Otago, PO Box 56, Dunedin, New Zealand. E-mail: jim.mann@stonebow.otago.ac.nz.

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the Cardiology Outpatients Clinic at Dunedin Public Hospital, Dunedin, New Zealand. None of the subjects was taking medication known to influence folate metabolism and all were non-smokers. All subjects gave informed consent and the study was approved by the Ethics Committee (Otago) of the Southern Regional Health Authority. All but 3 subjects completed the intervention; 1 withdrew because of unrelated ill health and 2 withdrew because of time constraints.

Study design

At recruitment, blood samples were collected in duplicate, 24–48 h apart, and subjects were asked to complete a 4-d diet record, which was used to assess usual food and nutrient intakes. Participants were then asked to follow a fat-modified diet [30% of energy as fat (10% as saturated fat), 50% as carbohydrate, and 20% as protein] for a 2-wk run-in period. Thereafter, dietary intake was reassessed, 2 additional blood samples were collected (baseline), and subjects were randomly assigned (after stratification by sex) to a control group or to 1 of 3 intervention groups: the dietary folate group (consumption of folate-rich foods), the cereal group (consumption of folic acid-fortified breakfast cereals), and the supplement group (consumption of folic acid supplements).

During the 12-wk intervention, 4-d diet records were completed and blood samples were collected at 6 and 12 wk. At each time point, 2 blood samples (after a 10–12-h fast) were collected 24–48 h apart for measurement of plasma homocysteine, lipids, and lipoproteins. Serum vitamin B-12 and folate, red blood cell folate, and plasma glucose, creatinine, and apolipoproteins A-I and B were measured in only one blood sample from each period. Fibrinogen was measured at recruitment and at the end of the intervention. Blood samples for homocysteine, cholesterol, and lipoprotein analyses were collected into EDTA-containing tubes, immediately placed on ice, separated within 2 h by centrifugation at $2000 \times g$ for 10 min at 4°C, and stored at –80°C until analyzed. Blood collected for serum vitamin B-12 and folate and red blood cell folate measurements was chilled and held in the dark until needed.

Diets

The control group continued to consume the fat-modified diet that was followed in the run-in period together with 5 mL canola oil/d as placebo. Subjects in the dietary folate group were instructed to increase their consumption of folate-rich foods so that their baseline intake of $\approx 250 \mu\text{g}$ folate/d increased to $\approx 600 \mu\text{g}/\text{d}$; they were provided with a list of folate-rich foods to select from (11), which also included information about the folate content of each food and suggested eating plans and recipes. The cereal group was asked to achieve the target intake of $600 \mu\text{g}$ folate/d by consuming 350–400 μg folate from commercially available folic acid-fortified cereals (fortified to contain 100 $\mu\text{g}/\text{serving}$). To achieve a folate intake approximately equal to that of the other 2 intervention groups, the supplement group was instructed to take 1.5 tablets of a folic acid supplement each day (300 μg folic acid/tablet; Blackmores Ltd, Balgowlah, Australia).

All subjects were given detailed dietary information by a registered dietitian at recruitment and randomization. This information was reinforced during fortnightly telephone interviews throughout the study and at the time of blood sampling. Additional encouragement and information were provided by tele-

phone or during individual counseling sessions when required. Compliance was assessed by analyzing 4-d diet records and by examining returned pill containers and daily checklists completed by subjects in the dietary folate and cereal groups. The nutrient composition of the foods listed on the 4-d diet records was determined by using DIET ENTRY & STORAGE and DIET CRUNCHER (12) and New Zealand food-composition data (13).

Laboratory methods

Cholesterol concentrations in plasma and lipoprotein fractions were measured enzymatically with kits and calibrators from Boehringer Mannheim (Mannheim, Germany), and triacylglycerol was measured enzymatically with kits from Roche Diagnostic Systems (Basel, Switzerland) on a Cobas Fara analyzer (Hoffmann-La Roche Inc, Nutley, NJ). After precipitation of lipoproteins containing apolipoprotein B with a phosphotungstate–magnesium chloride solution, HDL cholesterol was measured in the supernate (14). Apolipoproteins A-I and B were measured by immunoturbidimetry with kits from Boehringer Mannheim. CVs were 0.9% for cholesterol, 2.7% for triacylglycerol, 5.2% for HDL cholesterol, 6.1% for apolipoprotein A-I, and 10.2% for apolipoprotein B in the Royal Australasian College of Pathologists' quality-assurance program. LDL cholesterol was calculated by using the Friedewald formula (15). Serum vitamin B-12, serum folate, and red blood cell folate concentrations were determined by using a chemiluminescent method (ACS 180; Ciba-Corning, East Walpole MA) and had CVs of 4.3%, 10.9%, and 13%, respectively (internal quality-control program). Fibrinogen was determined by using a Sysmex CA 6000 Coagulation Analyser according to the method of Clauss (16) and had a CV of 4.7% (internal quality control). Glucose and serum creatinine were measured enzymatically with a Roche Diagnostic kit on a Cobas Fara analyzer and had CVs of 1.1% and 4.5%, respectively (internal quality control). Total homocysteine was determined by using the method of Ubbink et al (17) with cystamine dihydrochloride as internal standard (18). Reduced thiol derivatives were separated by using HPLC (LC-6A pumps with an SCL-6A system controller; Shimadzu Corp, Kyoto, Japan) on a 5- μm octadecyl silane 3, 10-nm (pore size) 150×4.6 mm column and 30×4.6 mm guard column (serial no. 137902, Prodigy; Phenomenex, Torrance, CA) and were detected by using fluorescence detection (Shimadzu RF551) with cystamine dihydrochloride (lot 76H2611; Sigma Chemical Co, St Louis) as the internal standard. The CV within batches was 2.2% and between batches was 5.3%. Mean values are given for variables measured on 2 occasions. Samples from each subject were analyzed in a single run to eliminate between-run variation. The folate content of all breakfast cereals was independently analyzed by KE Johnston in the laboratory of T Tamura (University of Alabama at Birmingham) with use of the trienzyme treatment method followed by the *Lactobacillus casei* microbiological assay (19). The cereal most frequently chosen by the participants contained 250 μg folic acid/100 g cereal; the product label indicated that the cereal contained 222 μg folic acid/100 g cereal, ie, the cereal provided 11% more folic acid than that stated by the manufacturer. However, because the amounts of folic acid in the less frequently chosen cereals varied and because we could not accurately quantify the amount of folic acid in the less frequently chosen cereals, our calculations of the folic acid content of the cereals are based on the manufacturer's claim of 100 $\mu\text{g}/\text{serving}$.

TABLE 1
Dietary characteristics of participants at recruitment and baseline

Detail	Recruitment (n = 65)	Baseline (n = 62)
Energy (MJ)	8.0 ± 1.9 ²	7.2 ± 1.9
Total fat (% of total energy)	31 ± 6 ²	27 ± 6
Fatty acids (% of total energy)		
Saturated	12 ± 3 ²	10 ± 3
Polyunsaturated	5 ± 2	5 ± 2
Monounsaturated	10 ± 2 ²	9 ± 2
Carbohydrate (% of total energy)	51 ± 6 ³	53 ± 7
Protein (% of total energy)	16 ± 3 ²	17 ± 4
Alcohol (% of total energy)	3 ± 4	3 ± 4
Folate (μg)	257 ± 93	272 ± 108
Vitamin B-6 (mg)	2 ± 1	2 ± 1
Vitamin B-12 (μg)	4 ± 3	4 ± 2

¹ $\bar{x} \pm SD$.

^{2,3}Significantly different from baseline: ² $P < 0.001$, ³ $P < 0.01$.

Statistical analysis

Data were log transformed when necessary to normalize the distribution, so geometric means and their 95% CIs are reported. All other data are reported as means ± SDs. Multiple regression analysis with use of dummy variables to compare the 3 treatment groups with the control group was used to determine the difference between the intervention and control groups, adjusted for baseline values. For log-transformed data, the difference between the intervention and control groups is expressed as a ratio (with 95% CIs). The percentage change during the intervention period was derived from the multiple linear regression. Product-moment correlations were used to determine associations between tHcy concentrations and biochemical (expressed as log-transformed data) and dietary variables. The selection of variables for product-moment correlations was based on those shown in previous studies to be significant determinants of tHcy concentrations. Variables that were significantly correlated with tHcy concentrations at recruitment and change in tHcy concentrations were included in a linear regression model to determine variance in tHcy concentrations and changes in tHcy concentrations. The reported dietary intakes of 4 subjects were excluded from the analysis determining variance of baseline homocysteine concentrations because of underreporting (20, 21). All reported dietary intakes were used in determining the explained variance in the final tHcy concentrations. All analyses were undertaken by using SPSS for WINDOWS release 6.1.3 (SPSS Inc, Chicago).

RESULTS

Characteristics of participants before randomization

Reported dietary intakes at recruitment and baseline (the time of randomization) are shown in **Table 1**. Of the various clinical and laboratory measurements, only the change in weight ($\bar{x} \pm SD$: 81.0 ± 12.0 kg at recruitment and 80.3 ± 12.0 kg at baseline), total cholesterol (5.90 ± 0.94 and 5.65 ± 0.93, respectively), and LDL cholesterol (3.89 ± 0.91 and 3.68 ± 0.84 mmol/L, respectively) were significantly different ($P \leq 0.01$) after the 2-wk run-in period. The mean tHcy concentration of the entire study group was 11.6 μmol/L (range: 6.9–23.8 μmol/L). The average daily intake of folate was well above the reference nutri-

ent intake (200 μg) and virtually all participants had concentrations of serum and red blood cell folate and serum vitamin B-12 within the respective reference ranges (5.7–45.0 nmol/L, 300–1750 nmol/L, and 160–600 pmol/L, respectively). None of the subjects had elevated concentrations of creatinine. After randomization, there were no significant differences in age, sex, or biochemical and dietary indexes between study groups.

Changes in homocysteine, blood folate, and serum vitamin B-12 concentrations

After 12 wk, tHcy decreased by 9%, 24%, and 21% in the dietary folate, cereal, and supplement groups, respectively, after adjustment for baseline values and the change in the control group (**Table 2**). The observed difference in the dietary folate group was not significant. The reduction in tHcy was greatest during the 0–6-wk period in all 4 groups. The continuing fall in tHcy from 6 to 12 wk, relative to the change in the control group, was significant in the cereal group ($P < 0.01$, regression analysis). Serum folate increased significantly in all 3 intervention groups. When compared with the changes in the control group, after adjustment for baseline values, there were similar large increases in serum folate in the cereal and supplement groups and a smaller increase in serum folate in the dietary folate group. The significant increase in serum folate was greatest in all 3 groups during the initial 6 wk, but the continued increase through 12 wk was also significant in all 3 groups. Only subjects in the cereal and supplement groups had a significant increase in red blood cell folate over the 12-wk intervention; the increase between 6 and 12 wk was significant only in the cereal and supplement groups. No significant change in serum vitamin B-12 was observed over the intervention period.

Reported dietary changes

Reported dietary folate, vitamin B-6, vitamin B-12, alcohol (% of total energy), and dietary fiber intakes during the intervention period are shown in **Table 3**. Over the 12-wk intervention period there was a significant increase in total folate intake in all 3 intervention groups; the largest increase was in the dietary folate group. Seventy percent of the dietary folate group achieved the target intake of 600 μg folate/d; the remaining 30% had an average intake of 498 μg folate/d. The main sources of dietary folate were yeast and yeast products (an average of ≈250 μg/d), cereals, cereal products, beans and nuts (≈245 μg/d from haricot beans, chickpeas, peanuts, and bread and bakery products), vegetables, fruit, and fruit juices (≈185 μg/d from asparagus, spinach, broccoli, broad beans, peas, potatoes, oranges, and orange juice). Only 30% of the cereal group achieved the target intake of 600 μg folate/d; the remaining 70% had an average intake of 424 μg/d. The average number of servings of cereals consumed during the intervention was 2.98/d. Ninety-eight percent of the supplement group consumed an additional 450 μg folic acid/d, resulting in an average intake of 662 μg/d. Vitamin B-6 intake increased over the 12-wk intervention in the dietary folate and cereal groups. No significant changes in vitamin B-12 intakes were observed. There was a significant reduction in the percentage of total energy from polyunsaturated (from 5% to 4%) and monounsaturated (from 10% to 8%) fatty acids in the cereal group during the intervention period (data not shown) and a significant increase in dietary fiber (from 30 to 38 g) in the dietary folate group but no other significant changes in total energy, macronutrients, or micronutrients.

TABLE 2Changes in total homocysteine (tHcy), serum folate, serum vitamin B-12, and red blood cell folate concentrations during the intervention period¹

	Baseline	Week 6	Week 12	Ratio ²	Adjusted difference ³
tHcy ($\mu\text{mol/L}$)					
Control group	12.3 (10.9, 14.0) ⁴	11.5 (10.0, 13.1)	11.6 (10.1, 13.3)	—	—
Dietary folate group	10.6 (9.4, 12.1)	9.7 (8.7, 10.8)	9.5 (8.3, 10.8)	0.91 (0.80, 1.03)	—
Cereal group	11.4 (9.9, 13.2)	9.1 (8.1, 10.4)	8.3 (7.2, 9.6)	0.76 (0.67, 0.86) ⁵	—
Supplement group	11.7 (10.4, 13.1)	9.0 (8.0, 10.1)	8.8 (7.8, 9.9)	0.79 (0.69, 0.89) ⁵	—
Serum folate (nmol/L)					
Control group	15 (12, 18)	13 (12, 15)	14 (12, 17)	—	—
Dietary folate group	15 (13, 17)	19 (17, 22)	22 (18, 26)	1.52 (1.28, 1.80) ⁵	—
Cereal group	18 (15, 22)	34 (29, 40)	39 (36, 43)	2.60 (2.19, 3.08) ⁵	—
Supplement group	15 (11, 20)	35 (31, 39)	42 (40, 44)	2.91 (2.46, 3.44) ⁵	—
Serum vitamin B-12 (pmol/L)					
Control group	242 (212, 277)	218 (185, 257)	236 (202, 275)	—	—
Dietary folate group	227 (187, 277)	198 (169, 232)	196 (165, 233)	0.86 (0.66, 1.12)	—
Cereal group	229 (186, 283)	219 (183, 261)	280 (209, 375)	1.22 (0.95, 1.58)	—
Supplement group	278 (229, 337)	231 (190, 282)	243 (194, 303)	0.95 (0.73, 1.23)	—
Red blood cell folate (nmol/L)					
Control group	539 \pm 166 ⁶	569 \pm 133	537 \pm 153	—	—
Dietary folate group	571 \pm 162	627 \pm 172	643 \pm 180	—	88.00 (–33.05, 209.05)
Cereal group	566 \pm 133	788 \pm 180	978 \pm 246	—	440.84 (319.89, 561.81) ⁵
Supplement group	594 \pm 209	808 \pm 173	991 \pm 160	—	423.63 (303.93, 543.34) ⁵

¹Control group, $n = 15$; dietary folate group, $n = 15$; cereal group, $n = 16$; supplement group, $n = 16$.²Ratio of geometric means of the intervention groups relative to the control groups at week 12, adjusted for baseline values.³Difference between intervention and control groups at week 12, adjusted for baseline values.⁴Geometric means; 95% CIs in parentheses.⁵ $P < 0.001$.⁶ $\bar{x} \pm \text{SD}$.

Determinants of homocysteine and serum folate concentrations at recruitment and during treatment

At recruitment, tHcy values were inversely associated with serum folate, serum vitamin B-12, dietary fiber, and dietary folate intakes (**Table 4**). No associations were observed between tHcy concentrations and creatinine, red blood cell folate, age, sex, cholesterol and lipoprotein concentrations, body weight, or any other dietary variable. Variables that were significantly correlated with tHcy concentrations at recruitment were entered into a regression model. After adjustment for age and sex, only serum folate contributed significantly to the variance in tHcy concentrations at recruitment ($R^2 = 0.19$). The change in tHcy concentrations during the intervention period was inversely correlated with the change in serum folate, red blood cell folate, and the percentage of total energy from alcohol at week 12, but not with other biochemical or dietary measures. Multiple regression suggested that after adjustment for age and sex, the change in serum folate accounted for 54% of the variance in the change in tHcy concentrations and the change in the percentage of total energy from alcohol accounted for 3% of the variance; no other dietary or metabolic variables accounted for any of the variance. At recruitment, serum folate correlated with dietary folate intake. The change in serum folate was significantly correlated with change in dietary folate.

Lipid, lipoprotein, and glucose concentrations and body weight

After the initial reduction in total cholesterol during the 2-wk run-in period, there were no further changes in total or LDL cholesterol. HDL cholesterol, triacylglycerol, apolipoproteins A-I and B, fibrinogen, and glucose did not change significantly during the intervention period. A small but significant weight gain

occurred during the 12-wk intervention in the dietary folate and cereal groups, from 78.06 to 78.58 kg ($P < 0.05$) and 77.47 to 78.42 kg ($P < 0.01$), respectively.

DISCUSSION

It has been known for some time that supplementation with 400 μg folic acid/d reduces tHcy concentrations in healthy individuals (8, 22). More recently, during a relatively short (5 wk) study, Malinow et al (9) observed reductions in tHcy of 11% and 14%, respectively, in IHD patients consuming cereals fortified with 499 and 665 μg folic acid/serving (9). Schorah et al (10) observed a 10% reduction in tHcy concentrations in healthy individuals consuming an additional 200 μg folic acid from cereals fortified with folic acid (10). However, this is the first study to compare the effects of similar amounts of additional folate derived from foods, or folic acid from commercially available fortified breakfast cereals and supplements on tHcy concentrations in free-living individuals. Compared with the change in the control group, there were 24% and 21% decreases in tHcy when subjects consumed an additional 298 μg folic acid/d from fortified cereals or 437 μg folic acid/d from supplements. The longer study period and overlapping CIs could explain the greater benefit resulting from breakfast cereals observed in our study compared with the findings of Malinow et al (9). However, the increases in serum and red blood cell folate were appreciably greater in the present study, suggesting better compliance by our participants. The difference in tHcy observed in the dietary folate group was not significant. Although there is no doubt that the response in this group was less impressive than that in the cereal and supplement groups, it is conceivable that the lower baseline concen-

TABLE 3Intakes of dietary folate (from foods, fortified cereals, and supplements), vitamin B-6, vitamin B-12, dietary fiber, and alcohol calculated from 4-d diet records¹

Nutrients and group	Baseline	Week 12	Adjusted difference ²
Dietary folate (μg)			
Food folate			
Control group	249 \pm 96 ³	227 \pm 68	—
Dietary folate group	289 \pm 99	707 \pm 238	—
Cereal group	315 \pm 133	219 \pm 122	—
Supplement group	234 \pm 88	224 \pm 91	—
Supplemental folic acid			
Control group	—	—	—
Dietary folate group	—	—	—
Cereal group	—	298 \pm 120	—
Supplement group	—	437 \pm 76	—
Total folate			
Control group	249 \pm 96	227 \pm 68	—
Dietary folate group	289 \pm 99	707 \pm 238	460.9 (354.6, 567.3) ^{4,5}
Cereal group	315 \pm 133	518 \pm 136	259.0 (152.9, 365.6) ⁵
Supplement group	234 \pm 88	662 \pm 103	442.3 (338.6, 546.0) ⁵
Vitamin B-6 (mg)			
Control group	1 \pm 1	1 \pm 1	—
Dietary folate group	2 \pm 1	3 \pm 1	1.2 (0.7, 1.8) ⁵
Cereal group	2 \pm 1	2 \pm 1	0.8 (0.2, 1.3) ⁶
Supplement group	1 \pm 1	1 \pm 1	0.0 (−0.5, 0.5)
Vitamin B-12 (μg)			
Control group	3 \pm 1	6 \pm 5	—
Dietary folate group	2 \pm 1	4 \pm 3	−1.7 (−4.9, 1.5)
Cereal group	5 \pm 3	6 \pm 6	0.8 (−2.5, 4.0)
Supplement group	3 \pm 2	3 \pm 1	−2.2 (−5.4, 0.9)
Alcohol (% of total energy)			
Control group	2 \pm 4	1 \pm 2	—
Dietary folate group	2 \pm 4	2 \pm 3	0.8 (−1.6, 3.3)
Cereal group	3 \pm 4	3 \pm 5	1.3 (−1.0, 3.7)
Supplement group	4 \pm 5	4 \pm 5	1.6 (−0.8, 4.0)
Dietary fiber (g)			
Control group	24 \pm 11	23 \pm 9	—
Dietary folate group	30 \pm 10	38 \pm 10	11.3 (5.6, 17.1) ⁵
Cereal group	28 \pm 9	24 \pm 7	−1.4 (−6.9, 4.1)
Supplement group	22 \pm 8	22 \pm 9	0.6 (−4.9, 6.1)

¹Control group, $n = 15$; dietary folate group, $n = 15$; cereal group, $n = 16$; supplement group, $n = 16$.²Difference between intervention and control groups at week 12, adjusted for baseline values.³ $\bar{x} \pm \text{SD}$.⁴Geometric means; 95% CIs in parentheses.⁵ $P < 0.001$.⁶ $P < 0.01$.

trations of tHcy may have reduced the tHcy-lowering potential of this dietary treatment. Nevertheless, our data provide strong evidence that folic acid fortification of breakfast cereals and the use of folic acid supplements are the most effective means of reducing tHcy concentrations.

The findings also provide some insights regarding the bioavailability of folate from various sources and the existence of a threshold intake above which no further benefit occurs. It appears that folic acid added to cereals is as available as is folic acid derived from supplements, suggesting that the cereal matrix has little effect on intestinal absorption (23, 24). Of interest are the similar changes in serum and red blood cell folate as well as in tHcy concentrations in the cereal and supplement groups, despite the fact that the cereal group achieved a total daily folate intake of 518 $\mu\text{g}/\text{d}$ and the supplement group achieved an intake of 662 $\mu\text{g}/\text{d}$. These folate intakes represent 298 and 437 μg

more, respectively, than provided in the diets of these 2 groups. Several groups have suggested a dose-response effect of supplemental folic acid on serum folate and tHcy concentrations, with a maximum effect being observed at intakes of $\approx 400 \mu\text{g}/\text{d}$ (1, 22, 25, 26). Because of the limited number of intakes tested, it is probably too early to draw firm conclusions.

Although the dietary folate group consumed appreciably more total folate than did the other 2 intervention groups, serum folate did not increase to the same extent. However, the extent of the increase in serum folate is comparable with that achieved in a recently published, short-term (4 wk) study investigating the effect of an increase in dietary folate consumption of 350 $\mu\text{g}/\text{d}$ (27). It has been estimated that as little as 50% of naturally occurring folate is available from a mixed diet (28). Different fruit and vegetables have different folate bioavailabilities. Citrus fruit and legumes, important sources of readily

TABLE 4

Variables correlated with total homocysteine (tHcy) and serum folate concentrations at recruitment and changes in tHcy and serum folate concentrations during the intervention period

Variable	Recruitment				Change in tHcy		Change in serum folate	
	tHcy		Serum folate		<i>r</i>	<i>(P)</i>	<i>r</i>	<i>(P)</i>
	<i>r</i>	<i>(P)</i>	<i>r</i>	<i>(P)</i>				
Serum folate ¹	-0.35	(<0.005)	—	—	—	—	—	—
Serum vitamin B-12 ¹	-0.26	(<0.05)	—	—	—	—	—	—
Dietary folate intake	-0.27	(<0.05)	0.25	(0.055)	—	—	—	—
Dietary fiber intake	-0.26	(<0.05)	—	—	—	—	—	—
Change in serum folate ¹	—	—	—	—	-0.66	(<0.0001)	—	—
Change in red blood cell folate	—	—	—	—	-0.32	(0.01)	—	—
Change in dietary folate intake	—	—	—	—	-0.25	(0.06)	0.40	(0.001)
Alcohol (% of total energy at week 12)	—	—	—	—	-0.41	(0.001)	—	—


¹Log-transformed data.

available folate (28), were relatively infrequent food choices by our free-living participants.

Many cross-sectional epidemiologic studies have examined determinants of tHcy concentrations in populations. Dietary and serum folate and serum vitamin B-12 have been consistently found to be inversely related to tHcy (29–31), with correlation coefficients that are consistent with those observed in the present study. However, at the time of recruitment we found that only 19% of the variance in tHcy concentrations could be explained, whereas in 2 other studies it accounted for up to 50% of the variance (30, 31). The inclusion in other studies of individuals potentially deficient in either folate or vitamin B-12, of individuals with a greater range of tHcy concentrations than those of the participants in the present study, and of the measurement of several additional variables could account for the apparent discrepancy. On the other hand, after adjustment for age and sex, change in serum folate explained 54% of the variance in the change in tHcy. The activity of methylenetetrahydrofolate reductase is considered to be one of the key factors determining tHcy concentrations and individual response to folate intervention (25). These observations provide clear evidence of the therapeutic potential of dietary intervention despite the powerful genetic determinants of the concentration and activity of this enzyme.

If the estimates by Boushey et al (1) are confirmed in randomized, controlled clinical trials, the 3- μ mol/L reduction in tHcy concentrations in the cereal and supplement groups could translate into a 30–40% reduction in IHD risk. The use of folic acid–fortified breakfast cereals and dietary supplements in tablet form appears to be the best means of achieving a reduction in IHD risk. The optimum folic acid fortification level in breakfast cereals remains to be estimated with certainty. However, the level of fortification used in the present study (100 μ g/serving) required consumption of more servings than would usually be consumed. The concentration of serum and red blood cell folate achieved was also sufficient to reduce the risk of congenital malformations (32). Further research regarding the bioavailability of folate-rich foods may enable more targeted dietary advice, which in turn would lead to a greater increase in serum folate and a more marked reduction in tHcy concentrations. However, it is clear that general advice to increase the consumption of folate-rich foods is not a particularly effective means of reducing tHcy concentrations.

Any advice, be it for individuals or populations, needs monitoring. The concentration of serum folate is often used as a measure

of folate status and as a surrogate measure of dietary intake. Given the analytic difficulties in determining the amount of folate in food, the resulting limitations of the folate data from the food-composition databases, and the variation in the bioavailability of folate in different foods, the correlation between total dietary folate and serum folate observed in this and in other studies (26, 33) almost certainly represents an underestimate of the association. This observation and the association between changes in serum folate and changes in tHcy concentrations suggest that the serum folate concentration is an appropriate indicator of changes in dietary intakes of folate and the likely clinical consequences. 

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