Dietary counseling to increase natural folate intake: a randomized, placebo-controlled trial in free-living subjects to assess effects on serum folate and plasma total homocysteine[1–3]

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ABSTRACT

Background: The association between vascular disease and elevated plasma total homocysteine (tHcy) concentrations is caused, in part, by inadequate intakes of dietary folate. Increasing folate intake either through supplements or foods naturally rich in folates has been shown to decrease tHcy concentrations.

Objective: The aim of this study was to determine whether a similar reduction in tHcy was possible in free-living persons receiving dietary counseling.

Design: The study included a 4-wk placebo-controlled dietary intervention trial in which participants consumed either unfortified breakfast cereal (control group) or an extra 350 μg folate derived from food/d (dietary group). Serum folate and tHcy concentrations in both groups were measured before and after the intervention period, and the concentrations in the dietary group were also measured 17 wk after the intervention period.

Results: During the 4-wk intervention, mean dietary folate intake in the dietary group increased from 263 (95% CI: 225, 307) to 618 μg/d (535, 714), resulting in a mean increase in serum folate of 37% (15%, 63%) and a decrease in tHcy from 12.0 (10.9, 13.3) to 11.3 μmol/L (10.2, 12.5). A further decrease in tHcy occurred in the dietary group during follow-up, with a final tHcy concentration of 9.7 μmol/L (8.8, 10.8).

Conclusions: Increasing natural folate intake improved folate status and decreased tHcy concentrations to an extent that may significantly reduce the risk of vascular disease. Dietary modification may have advantages over folic acid fortification because the altered food-consumption patterns lead to increased intakes of several vitamins and minerals and decreased intakes of saturated fatty acids. *Am J Clin Nutr* 2002;76:758–65.

KEY WORDS Folate, folate intake, homocysteine, diet, dietary counseling, randomized trial, community participants

INTRODUCTION

The association of moderately elevated concentrations of plasma total homocysteine (tHcy) with vascular disease has led to research on ways of decreasing tHcy concentrations (1). Many studies have shown that folic acid, given either as a supplement or through fortification, is effective in decreasing tHcy concentrations (2); fewer studies have examined the possibility of decreasing tHcy concentrations by increasing the intake of foods that are naturally rich in folate. In addition, little attention has been given to the potential of folate-rich foods to reduce the risk of neural tube defects (NTDs), although a reduction in occurrent NTD risk associated with dietary intake has been reported (3). The potential of dietary change rather than fortification or supplementation is an important issue because changes in dietary habits may well result in additional health benefits and because some concerns remain, especially in the elderly, regarding high levels of folic acid fortification (4). In tightly controlled feeding experiments, dietary changes significantly decreased tHcy concentrations (5–7). However, free-living individuals who were advised to increase their intake of folate-rich foods were unable to significantly decrease their tHcy concentrations (8). In this randomized, placebo-controlled study, we advised free-living individuals to increase their consumption of foods rich in bioavailable folate to examine the effects on serum folate and tHcy concentrations.

SUBJECTS AND METHODS

Subjects

Subjects were recruited in Dunedin, New Zealand by advertising in local newspapers during June–September 1999. Respondents were ineligible to participate in the study if they were taking anticonvulsant drugs or fibrates or were receiving hormone replacement therapy; were pregnant; or had been diagnosed with HIV, hepatitis, or pernicious anemia. Participants were considered eligible for the study if they were ≥18 y of age and had fasting plasma tHcy concentrations ≥10 μmol/L. This tHcy concentration was chosen because it represented the cutoff for the upper quartile of distribution in our population. We believed that persons at the upper end of the normal range would respond to folate intervention to a greater extent than would persons with lower tHcy concentrations and that persons with higher concentrations would potentially benefit the most from a reduction in tHcy concentration. As a result of screening

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[2] Unfortified breakfast cereal and partial funding of the study were provided by Kellogg (Australia) Pty Ltd.

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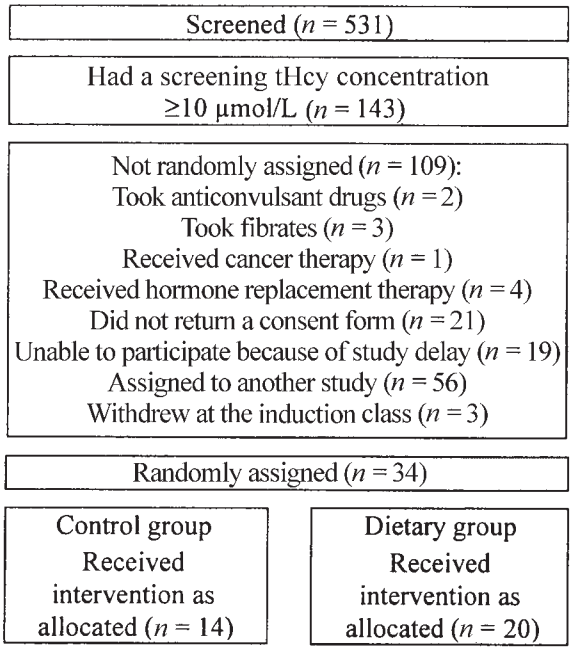

FIGURE 1. Flowchart showing the progress of respondents from initial screening through the randomized trial. Fifty-six persons were randomly assigned to another study, in which the effects of fortified breakfast cereals were examined. tHcy, total homocysteine.

blood tests, 143 persons with a tHcy concentration ≥ 10 μmol/L were identified. Nineteen persons were unable to participate because the start date of the study was delayed until February 2000. The numbers of other volunteers who were excluded or who withdrew are shown in **Figure 1**. After all exclusions and withdrawals, there were 34 participants (15 women and 19 men) who were randomly assigned to either the dietary group or the control group. None of the participants reported using supplements known to contain folic acid. The University of Otago Human Ethics Committee approved the study, and the subjects provided written, informed consent.

Protocol

At recruitment, 2 wk before the start of the intervention (week −2), a registered dietitian instructed participants to follow the recommendations for healthy eating presented in the National Heart Foundation of New Zealand's *Food Guide* (9). Note that instruction was given before the baseline blood measurements were taken to ensure that all participants began the study with a reasonably similar dietary intake. Relatively few foods, chiefly breakfast cereals, are fortified in New Zealand. Participants were advised to avoid eating foods fortified with folic acid and were provided with a list of unfortified brands. Participants were supplied with electronic kitchen scales accurate to 2 g and were instructed on how to complete a weighed food record; the first 4-d record was completed before baseline. At the start of the study (week 0), subjects in the dietary group received individual instruction on ways to increase their dietary folate intake to reach a target of 600 μg folate/d. Subjects were recommended to consume 2 glasses of orange juice/d and 1 serving of legumes/d and to then self-select items from a list of folate-rich foods for the next 4 wk. Each subject received an allowance of NZ$20/wk. The control group was given unfortified breakfast cereal and instructed to weigh and eat 20 g of cereal/d (providing 16 μg folate/d); compliance was determined from a daily checklist.

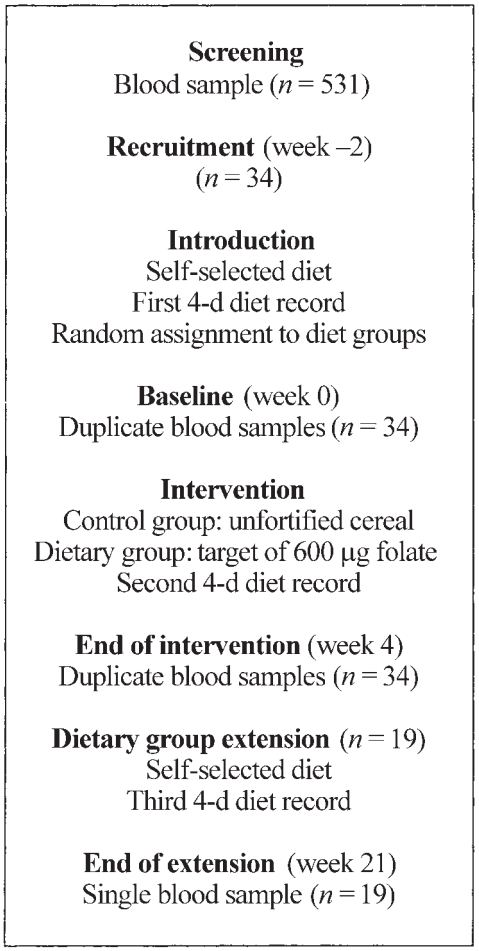

FIGURE 2. Study schedule and timing of data collection.

Duplicate fasting blood samples, which were drawn 48 h apart, were collected at baseline and at the end of the intervention. Concentrations of lipids, lipoproteins, and tHcy were measured in all blood samples. Serum folate and serum vitamin B-12 concentrations were measured in one blood sample at week 0 and week 4, and concentrations of plasma glucose and creatinine were measured at baseline. After the 4-wk randomized, controlled trial, 19 of the 20 subjects in the dietary group agreed to continue to avoid eating fortified products for the next 17 wk, a third diet record was completed at week 19, and a single fasting blood sample was drawn at week 21. The purpose of this follow-up period was to determine the extent to which the dietary practices established in the intervention period were likely to be sustained and whether any effect on tHcy concentration was maintained. The sequence and timing of data collection are shown in **Figure 2**.

Outcome measures

The primary outcome measures were changes in tHcy and serum folate concentrations in the dietary group compared with those in the control group. Plasma tHcy concentrations are dependent on many factors including nutritional and genetic factors and disease (10). Major dietary determinants are the vitamins folate, B-6, and B-12 (11). Dietary data were therefore collected before and during the intervention to assess any dietary changes that may have affected the primary outcome variables. A common polymorphism in folate and homocysteine

TABLE 1
Baseline characteristics of the control and dietary groups[1]

	Control group (n = 14)	Dietary group (n = 20)
Age (y)[2]	60 ± 14.5	58 ± 17.5
Male (%)	57	55
Weight (kg)	74 (68, 81)[3]	74 (69, 79)
Plasma total cholesterol (mmol/L)	5.1 (4.7, 5.6)	5.1 (4.7, 5.7)
Plasma glucose (mmol/L)	5.4 (5.1, 5.8)	5.2 (5.0, 5.4)
Plasma creatinine (μmol/L)	73 (67, 78)	72 (64, 80)
Dietary vitamin B-6 (mg/d)	1.6 (1.3, 2.0)	1.6 (1.3, 1.9)
Dietary methionine (g/d)	1.7 (1.5, 1.9)	1.6 (1.4, 1.7)
MTHFR genotype (677C→T polymorphism)		
CC	5	11
CT	8	5
TT	1	4

[1] MTHFR, 5,10-methylenetetrahydrofolate reductase. There were no significant differences between the groups.
[2] Arithmetic $\bar{x}$ ± SD.
[3] Geometric $\bar{x}$; 95% CI in parentheses.

metabolism is a 677C→T point mutation in the gene encoding 5,10-methylenetetrahydrofolate reductase (MTHFR; EC 1.7.99.5) that influences tHcy concentrations when plasma folate concentrations are low (12). tHcy concentrations also increase during renal failure, which is closely related to serum creatinine concentrations (13). Thus, genotyping and measurement of plasma creatinine were carried out to control for these variables.

Randomization

Names were written onto cards, stratified according to sex, and allocated to a group by simple randomization. All subjects were randomly assigned at the same time.

Laboratory methods

The total folate content of the unfortified breakfast cereal was determined with the use of a microbiological assay with *Lactobacillus casei* and trienzyme extraction (14) at the Department of Food Science and Technology, University of New South Wales, Australia. Blood samples for tHcy, lipids, glucose, and creatinine measurements were collected in EDTA-coated tubes and placed on ice immediately. Plasma was separated within 2 h by centrifugation at 2000 × *g* for 10 min at 4 °C and stored at −20 °C until analysis. Serum folate and vitamin B-12 concentrations were measured with the use of a chemiluminescence method (ACS180; Ciba Corning, East Walpole, MA); the CVs were 10.9% and 4.3%, respectively. Plasma tHcy concentrations were measured with the use of HPLC with fluorescence detection by adapting the method of Ubbink et al (15) and by using cystamine dihydrochloride as an internal standard (16). One pooled sample was inserted for every 10 samples, and the CVs within and between batches were 6.7% and 6.9%, respectively. The 4 samples from each subject (2 at baseline and 2 after the intervention) were analyzed in a single run to eliminate between-run variation. The total cholesterol concentration in plasma and lipoprotein fractions was measured enzymatically with kits and calibrators from Roche Diagnostics GmbH (Mannheim, Germany) on a Cobas Fara analyzer (Roche Diagnostics Systems, Basel, Switzerland); the CV was 0.9%. Plasma glucose and creatinine concentrations were measured enzymatically with diagnostic kits from Roche Diagnostics GmbH on a Cobas Fara analyser (CVs: 1.1% and 4.5%, respectively).

Identification of the 677C→T polymorphism in the gene encoding MTHFR was conducted after extraction of DNA from blood samples by adaptation of published methods (17, 18). The upstream primer, 5′AGGGAGCTTTGAGGCTGACCTGAA, and downstream primer, 5′GGGGACGATGGGGCAAGTGAT, were designed to amplify a 151–base pair fragment bracketing the polymorphism. Polymerase chain reaction was performed with final concentrations of 10 ng DNA/μL, 0.2 μmol dNTP/L, 250 nmol of each primer/L, 10X PCR Buffer (Qiagen, Hilden, Germany), and 0.025 U Taq/μL (Qiagen) in a 20-μL reaction. The thermal cycler (PCR Express; Thermo Hybaid, Ashford, United Kingdom) was set to denature for 3 min at 94 °C, followed by 35 cycles each at 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 20 s, with a final 2-min extension at 72 °C. *Hin*fI (New England Bio Labs, Beverly, MA) was diluted to 0.66 U/μL according to the manufacturer's instructions, and 2 U was added to each sample reaction. The plate was incubated at 37 °C for 120 min to ensure complete digestion of the *T* allele into 101– and 50–base pair fragments; the *C* allele was unaltered. Samples were electrophoresed on an agarose gel, stained with ethidium bromide, and viewed under ultraviolet light. Two independent observers classified each sample into 1 of 3 genotypes: *CC*, *CT*, or *TT*.

Dietary analysis

The US Department of Agriculture food composition database was used to provide the methionine content of foods (19). All other nutrient values were based on New Zealand food composition data (20), and the diet records were analyzed with the use of DIET CRUNCHER software (21).

Statistical analysis

Where appropriate, data were log transformed to normalize the distribution and back transformed to geometric means with 95% CIs. The distribution of *MTHFR* genotypes in the 2 groups was compared with the use of Fisher's exact test, and other baseline characteristics were compared with the use of Student's *t* test. Multiple regression was used to estimate the difference between the control and dietary groups after adjustment for baseline values. For log-transformed data, the differences between the dietary and control groups are expressed as ratios (with 95% CIs). Ratios are statistically significant if the CIs do not include 1. After log transformation, nutrient intake values for the baseline diets of the control and dietary groups were compared with the use of Student's *t* test. After log transformation, the tHcy concentration after the 17-wk extension period was compared with the baseline value by using a paired *t* test. Changes in food consumption were compared with the use of Wilcoxon's signed rank test. The analyses were undertaken with the use of SPSS for WINDOWS release 6.1.3 (SPSS Inc, Chicago).

RESULTS

The baseline characteristics of the control and dietary groups are shown in **Table 1**. Mean (± SD) baseline tHcy concentrations were 12.4 ± 3.3, 14.6 ± 3.9, and 11.7 ± 1.4 μmol/L for the *CC*, *CT*, and *TT* genotypes, respectively. The results of the intervention are shown in **Table 2**. Dietary intakes and serum concentrations of vitamin B-12 after the 4-wk intervention were not significantly different from those at baseline in either group. After the intervention, folate intake in the dietary group was 2.6-fold higher (95% CI: 2.2, 3.1) than that in the control group, and serum folate and plasma tHcy concentrations in the dietary group were 37% higher (95% CI: 15%, 63%) and 10% lower (95% CI:

TABLE 2
Results of the 4-wk placebo-controlled intervention trial[1]

Variable	Baseline	After 4-wk intervention	Ratio of dietary to control[2]
tHcy (μmol/L)			
Control group (n = 14)	13.8 (11.9, 16.0)	14.1 (12.3, 16.2)	—
Dietary group (n = 20)	12.0 (10.9, 13.3)	11.3 (10.2, 12.5)	0.90 (0.85, 0.96)[3]
Serum folate (nmol/L)			
Control group (n = 14)	18 (15, 22)	18 (16, 21)	—
Dietary group (n = 20)	18 (16, 19)	25 (21, 28)	1.37 (1.15, 1.63)[3]
Serum vitamin B-12 (pmol/L)			
Control group (n = 14)	209 (178, 246)	217 (187, 252)	—
Dietary group (n = 20)	253 (229, 280)	273 (248, 301)	1.10 (0.98, 1.23)
Dietary folate (μg/d)			
Control group (n = 14)	272 (242, 307)	242 (215, 273)	—
Dietary group (n = 20)	263 (225, 307)	618 (535, 714)	2.6 (2.2, 3.1)[3]
Dietary vitamin B-12 (μg/d)			
Control group (n = 14)	4.4 (3.1, 6.2)	4.5 (3.6, 5.5)	—
Dietary group (n = 20)	3.3 (2.4, 4.6)	4.4 (2.6, 7.4)	1.02 (0.53, 1.95)

[1] Geometric $\bar{x}$; 95% CI in parentheses. tHcy, total homocysteine.
[2] At week 4 after adjustment for baseline values.
[3] $P < 0.05$.

4%, 15%), respectively, than those in the control group. In the dietary group, mean tHcy concentrations decreased from 12.0 μmol/L (95% CI: 10.9, 13.3) at baseline to 9.7 μmol/L (95% CI: 8.8, 10.8) after the 17-wk follow-up period ($P < 0.01$). During the 17-wk follow-up period in the dietary group, folate intake decreased from 618 (95% CI: 535, 714) to 393 μg/d (95% CI: 339, 455), and serum folate concentrations decreased from 25 (95% CI: 21, 28) to 21 nmol/L (95% CI: 17, 24), although both dietary and serum folate values after the 17-wk follow-up period remained higher than those at baseline. Adjusting for plasma creatinine concentration or *MTHFR* genotype in multiple regression analysis did not add to the explanatory power of the model. Plasma total cholesterol concentrations after the dietary intervention were 5.8 (95% CI: 5.4, 6.3) and 5.4 mmol/L (95% CI: 4.7, 6.2) in the dietary and control groups, respectively.

Average nutrient intakes before randomization were not significantly different between the subjects who were subsequently assigned to the dietary group and those who were assigned to the control group. The average nutrient intake of the control group remained unchanged despite the daily inclusion of 20 g of unfortified breakfast cereal during the study. Dietary counseling resulted in significant differences between the dietary and control groups in the intakes of several nutrients (**Table 3**). After the intervention, total energy intake and the percentage of energy from total fat and

TABLE 3
Dietary analysis of average daily intakes of nutrients during the 4-wk intervention period[1]

Variable	Dietary group (n = 20)	Control group (n = 14)	Ratio of dietary to control
Energy (kJ)	8270 (7260, 9350)	7820 (6760, 9040)	1.11 (0.93, 1.34)
Percentage of energy			
Total fat (%)	25 (23, 29)	26 (21, 31)	0.97 (0.82, 1.15)
SFAs (%)	7 (6, 9)	10 (8, 13)	0.71 (0.57, 0.87)[2]
PUFAs (%)	6 (5, 7)	4 (3, 5)	1.49 (1.16, 1.91)[2]
MUFAs (%)	9 (8, 10)	9 (7, 11)	1.02 (0.82, 1.25)
Protein (%)	18 (17, 19)	16 (15, 17)	1.11 (1.02, 1.21)[2]
Carbohydrate (%)	55 (52, 58)	54 (50, 58)	1.00 (0.93, 1.09)
Fiber (g)	36 (31, 42)	23 (19, 27)	1.61 (1.30, 1.99)[2]
Calcium (mg)	940 (780, 1120)	720 (570, 910)	1.15 (0.92, 1.44)
Iron (mg)	16 (14, 19)	11 (10, 13)	1.47 (1.23, 1.75)[2]
Sodium (mg)	2200 (1880, 2580)	2800 (2290, 3420)	0.79 (0.60, 1.04)
Total vitamin A (μg)[3]	1490 (960, 2310)	1150 (970, 1380)	1.34 (0.78, 2.32)
Thiamin (mg)	2.1 (1.8, 2.4)	1.6 (1.5, 1.8)	1.35 (1.12, 1.64)[2]
Riboflavin (mg)	2.3 (2.0, 2.7)	1.9 (1.7, 2.2)	1.19 (0.97, 1.47)
Vitamin B-6 (mg)	2.5 (2.1, 3.1)	1.8 (1.5, 2.2)	1.39 (1.09, 1.79)[2]
Vitamin B-12 (μg)	4.3 (2.6, 7.3)	4.4 (3.6, 5.5)	1.01 (0.52, 1.96)
Vitamin C (mg)	363 (285, 463)	84 (66, 107)	3.93 (3.05, 5.07)[2]
Vitamin E (mg)	16 (12, 20)	8.6 (7.2, 10.2)	1.87 (1.43, 2.44)[2]
Total niacin (mg)[4]	43 (38, 49)	35 (31, 40)	1.32 (1.11, 1.58)[2]
Folate (μg)	618 (535, 714)	242 (215, 273)	2.59 (2.19, 3.08)[2]

[1] Geometric $\bar{x}$; 95% CI in parentheses. SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid.
[2] $P < 0.05$.
[3] Micrograms retinol plus one-sixth times micrograms β-carotene equivalents.
[4] Milligrams preformed niacin plus one-sixth times milligrams tryptophan.

TABLE 4
Amount of folate obtained from various food items consumed by subjects in the dietary group (n = 20) before and after dietary counseling[1]

Food item	Introductory diet period	Intervention diet period
	μg folate/d	
Vegetables	60 ± 36.2	114 ± 44.6[2]
Legumes	12 ± 16.6	92 ± 66.0[2]
Cereals	39 ± 50.3	90 ± 65.4[2]
Orange juice	6 ± 6.5	82 ± 18.8[2]
Fruit	24 ± 15.9	54 ± 30.1[2]
Nuts and seeds	4 ± 8.6	35 ± 37.5[2]
Bakery	28 ± 19.7	30 ± 22.4
Yeast products	21 ± 52.3	30 ± 43.0
Liver and kidney	2 ± 5.6	25 ± 65.3
Dairy	19 ± 12.2	21 ± 12.7
Rice and pasta	12 ± 6.3	16 ± 3.2
Meat	7 ± 6.6	6 ± 4.4
Tea	5 ± 3.9	4 ± 3.2
Restaurant meals	4 ± 10.0	4 ± 4.8
Seaweed	4 ± 20.2	4 ± 19.4
Eggs	6 ± 8.9	3 ± 4.8
Soup	2 ± 5.4	3 ± 5.6
Fast food	3 ± 1.9	2 ± 5.2
Fish	2 ± 3.8	2 ± 5.8
Snacks	3 ± 5.5	1 ± 5.7

[1] $\bar{x}$ ± SD. None of the foods contained folic acid.
[2] Significantly different from the introductory diet period, $P \leq 0.001$.

carbohydrate were not significantly different between the 2 groups. However, the percentages of energy from saturated fatty acids and polyunsaturated fatty acids were 29% lower (95% CI: 13%, 43%) and 49% higher (95% CI: 16%, 91%) in the dietary group than in the control group. Intakes of fiber, iron, and most vitamins during the intervention were higher in the dietary group than in the control group. In the dietary group, nutrient intake during the 17-wk follow-up period was intermediate between that recorded during the introductory and intervention phases (data not shown). At baseline and after the intervention, mean body mass was 73.6 (95% CI: 68.7, 78.8) and 73.4 kg (95% CI: 68.4, 81.4), respectively, in the dietary group and 74.5 (95% CI: 68.5, 78.7) and 74.2 kg (95% CI: 67.9, 81.1), respectively, in the control group.

For the dietary group, the amount of folate obtained from various food items is shown in **Table 4**. Increased intakes of vegetables, legumes, cereals, orange juice, fruit, and nuts and seeds accounted for most of the additional folate consumed during the intervention period. The total quantity of fruit eaten was similar between the 2 dietary periods, but fruit with a low folate content, eg, apples, peaches, and plums, was replaced with oranges and bananas, which contain more folate. Changes in the consumption of individual foods after dietary instruction are shown in **Table 5**. More beans, orange juice, asparagus, broccoli, nuts and seeds, chickpeas (garbanzo beans), oranges, green peas, and muesli were consumed by more subjects, and some subjects introduced avocado, yeast spread, and spinach into their diets. Most subjects in the dietary group ate more bananas after dietary instruction, and 1 subject introduced liver into the diet. In general, intakes of dairy products, eggs, and fish remained constant, but the intake of meat decreased somewhat.

DISCUSSION

When consumption of folate-rich foods increased to 600 μg/d after dietary counseling, serum folate concentrations increased significantly and plasma tHcy concentrations decreased significantly. After the conclusion of the randomized, controlled trial, tHcy concentrations decreased further during the next 17 wk, when no target folate intake was set. The continuing decrease occurred despite the fact that dietary folate intake decreased somewhat from that at the conclusion of the intervention period. The reason for the further decrease in tHcy concentration may have been due to a higher long-term daily folate intake, which increased

TABLE 5
Amount of foods consumed per day before and after dietary counseling[1]

Food	Introductory diet period	Number of consumers	Intervention diet period	Number of consumers	Number of new consumers	P
	g		*g*			
Beans	42 ± 31.5[2]	6	93 ± 61.4	19	13	0.000
Orange juice	137 ± 123.3	7	522 ± 241.6	19	12	0.000
Asparagus	0 ± ND	0	29 ± 8.5	9	9	0.008
Broccoli	24 ± 11.5	7	42 ± 37.1	16	9	0.022
Nuts and seeds	8 ± 8.2	8	37 ± 34.4	17	9	0.000
Chickpeas	23 ± ND	1	24 ± 12.3	8	7	0.028
Oranges	34 ± 15.8	8	108 ± 33.7	14	6	0.001
Green peas	15 ± 6.4	13	33 ± 23.0	18	5	0.002
Muesli[3]	40 ± 36.2	13	60 ± 34.9	18	5	0.002
Avocado	36 ± 31.5	2	24 ± 12.9	7	5	0.066
Yeast spread[4]	4 ± 2.8	5	4 ± 3.1	10	5	0.061
Spinach	24 ± 16.0	10	18 ± 10.9	13	3	0.889
Bananas	55 ± 32.4	15	84 ± 49.6	17	2	0.020
Liver and kidney	21 ± 5.1	2	54 ± 20.5	3	1	0.225

[1] n = 20. ND, not determined.
[2] $\bar{x}$ ± SD.
[3] Oat-based breakfast cereal containing dried fruits, nuts, and seeds.
[4] A savory spread used on toast or in sandwiches.

from 263 μg/d at baseline to 618 μg/d during the intervention period and was maintained at ≈400 μg during the 17 wk of continuing observation. In the absence of a control group during the follow-up period, it is conceivable that the continuing decrease in tHcy concentration was due to regression to the mean. However, the absence of a downward trend in the control group during the first 4 wk and the consistency of tHcy concentrations in the control groups of longer-term studies argue against this possibility (8, 22). The folate-rich diet significantly decreased the intake of saturated fatty acids by the dietary group. This decrease was not accompanied by significant changes in blood concentrations of total cholesterol, possibly because dietary instruction aimed at reducing fat intake was given to all participants (control and dietary groups) in the period before the baseline blood samples were taken.

Studies in which strictly controlled feeding conditions were used have reported significant decreases in tHcy concentration after increases in dietary folate. Brouwer et al (5) provided meals containing ≈560 μg folate for 4 wk, during which time plasma and red cell folate concentrations increased and tHcy concentrations decreased. Broekmans et al (6) compared 2 groups, one of which was provided with 100 *g* fruit and vegetables/d (low) and the other with 500 *g* fruit and vegetables/d plus 200 *g* fruit juice/d (high). After 4-wk a comparison of the 2 groups showed that plasma folate concentrations were higher and tHcy concentrations lower in the high group than in the low group. Appel et al (7) found that subjects who consumed a diet high in fruit and vegetables and low in fat for 11 wk had serum folate and tHcy concentrations that were significantly different from those of subjects who consumed a control diet. Through dietary counseling of free-living subjects, Riddell et al (8) increased dietary folate intake > 400 μg/d. Serum folate concentrations increased significantly, but the modest increase in red blood cell folate concentrations and the decrease in tHcy concentrations were not significant. The authors suggested that the lack of response may have been due to a relatively low baseline concentration of tHcy (10.6 μmol/L), making a decrease in concentration unlikely. Another possibility is the source of dietary folate. Yeast and yeast products accounted for a substantial proportion of the folate intake, ≈250 μg/d. This source of folate has been shown to have variable and usually low bioavailability (23, 24).

It is generally accepted that folic acid fortification is a more appropriate method of lowering tHcy concentrations and of reducing the risk of NTDs than is recommending increases in dietary folates (8, 25). Folic acid, an oxidized monoglutamyl folate, has higher bioavailability than do the reduced polyglutamylated folates that occur naturally in foods (26); the difference is attributed to several dietary and physiologic factors (27). Furthermore, compliance is easier to achieve with folic acid under study conditions because precise amounts can be taken as supplements or in fortified foods. In contrast, measurement of dietary folate intake is imprecise because amounts of food are frequently estimated rather than measured, the accuracy of food composition tables is variable (28), and characterization of folate intakes requires several days of recording (29, 30). However, when Brouwer et al (5) controlled dietary folate intakes by analyzing whole meals for folate content and requiring that meals be eaten under supervision, they found that natural folates increased red blood cell folate concentrations to the same extent as did folic acid. Despite this, they also found that natural folates were less effective than was folic acid at decreasing tHcy concentrations over 4 wk. In our dietary group, tHcy concentrations continued to decrease between weeks 4 and 21 despite a somewhat reduced intake of folate-rich foods.

Studies involving folic acid supplementation suggest that most of the decrease in tHcy concentration occurs within the first few weeks of supplementation (5, 8). In the present study, tHcy concentrations continued to decrease beyond 4 wk. This suggests that plasma tHcy concentrations may respond more quickly to folic acid than to natural folates. There is at least some evidence to support such a notion. Reduced folates are largely methylated across the intestinal mucosa (31), whereas some orally administered folic acid passes into the portal blood supply unaltered (32). Animal studies have shown that folic acid is taken up and retained by the liver better as 5-methyltetrahydrofolate pentaglutamate than as reduced folates, which, after intestinal methylation, may have to be demethylated by peripheral tissue and transported back to the liver before hepatic retention occurs (33, 34). An increase in the hepatic folate pool of 5-methyltetrahydrofolate pentaglutamate [a preferred substrate for methionine synthase (EC 2.1.1.13)] (35) would increase the intracellular concentration of *S*-adenosylmethionine, a promoter of cystathionine β-synthase (EC 4.2.1.22) on the transsulfuration pathway (36), which is the main catabolic route for homocysteine. Because the liver is a major exporter of circulating homocysteine (37), this may explain why folic acid appears to be more effective in the short-term at decreasing tHcy concentrations than are natural folates. Our data show that serum folate concentrations increased to 25 nmol/L in the dietary group over 4 wk and were maintained at > 20 nmol/L during the follow-up period. Serum folate concentrations in this range are considered adequate to prevent elevations in tHcy concentrations (38, 39). Intervention trials are required to determine with certainty whether an elevated plasma tHcy concentration causes the development of vascular disease or is simply a marker of atherogenesis (40). However, if a causal link is confirmed, increasing the intake of folate-rich foods may be an alternative or adjunct strategy to folic acid fortification.

Increasing the intake of vegetables, fruit, cereals, and nuts is likely to lead to positive changes in the intake of other important nutrients and is in agreement with dietary guidelines in all countries. Indeed, other vitamins involved with homocysteine metabolism may have contributed to the decrease in tHcy concentrations reported in the present study. The intake of vitamin B-6 in the dietary group increased ≈0.7 mg/d during the intervention period. tHcy concentrations in a group of subjects who were folate (supplemented with folic acid) and riboflavin replete decreased 7.5% with supplementation of 1.6 mg vitamin B-6/d for 12 wk (41). In contrast, tHcy concentrations after daily supplementation with 0.5 mg pyridoxine, contained in a multivitamin preparation that included riboflavin, for 24 wk were not significantly lower than those after supplementation with folic acid alone (22). However, it is possible that some of the decrease in tHcy concentration observed in the present study may have been attributable to the effects of increased intakes of vitamin B-6 and riboflavin.

Serum folate concentrations in the range achieved by dietary modification in the present study are also associated with a low risk of NTDs (42). Epidemiologic data from China provide confirmation of the effectiveness of natural folates. Without supplementation, the rate of NTDs in pregnancies of ≥ 20-wk duration was 6.5/1000 pregnancies in the north and 0.8/1000 pregnancies in the south (43). Because the higher rate of NTDs in the north was folate-responsive to 400 mg folic acid/d and the populations were ethnically homogeneous, the authors suggested that the background rates of NTDs could be attributable in part to differences in dietary folate intake. Such dietary differences

between northern and southern Chinese provinces have been reported (44).

Thus, natural folates have the potential to improve folate status to an extent that appreciably decreases tHcy concentrations and probably the risk of NTDs, although these benefits may take a little longer to accrue with natural folates than with folic acid. An important consideration is the need to ensure that the food sources are rich in bioavailable folate. A diet that is rich in natural folates is likely to confer additional benefits by virtue of its lower content of saturated fatty acids and its higher content of various vitamins and minerals. It is acknowledged that improved folate status as a result of dietary change requires substantial modification to present Western dietary habits and that full compliance may be difficult to achieve. Because a relatively small amount of additional folic acid may produce the required effect (45, 46), a complementary approach to improving folate nutrition by combining low-dose fortification with a persistent strategy of public education may produce maximal public health benefits.

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