Dietary Intake Pattern Relates to Plasma Folate and Homocysteine Concentrations in the Framingham Heart Study[1,2]

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ABSTRACT We examined the relationship between intake of food group (and supplement) sources of folate and plasma folate and homocysteine concentrations among 885 elderly subjects in the Framingham Heart Study. Dietary data were collected by food-frequency questionnaire, and blood samples analyzed for folate and homocysteine concentrations. Top contributors to total folate intake were ranked. Mean folate intake, plasma folate and homocysteine concentrations were estimated for users vs. non-users of supplements, and key foods—those which both contribute to total folate intake and are known to be good sources of folate—and examined statistically with adjustment for age, gender and total energy intake. Plasma folate and homocysteine concentrations were also determined by quintile of intake frequency for breakfast cereals and for fruits and vegetables. Plasma folate was significantly greater and homocysteine lower in women than in men. Despite somewhat greater plasma folate concentrations with age, homocysteine was significantly higher in those over 80 y of age than in younger subjects. Major contributors to folate intake were cold breakfast cereals (13.3%), multivitamins (12.8%) and orange juice (12.4%). Users of supplements, breakfast cereals, or green leafy vegetables had significantly greater plasma folate and lower homocysteine levels than non-users. Plasma folate concentration was also greater in those who drank orange juice. We identified clear dose-response relationships for both plasma folate and homocysteine with increased quintile of breakfast cereal and of fruit and vegetable use. Frequent consumption of these foods is associated with higher folate and lower homocysteine concentrations. J. Nutr. 126: 3025–3031, 1996.

INDEXING KEY WORDS:

- *folate* • *homocysteine* • *elderly humans*
- *breakfast cereal* • *fruits and vegetables*

The importance of folate to health is gaining increasing recognition. In response to the serious problem of neural tube defects, a recommendation has been made for all women of child bearing age to consume 400 μg of folate per day (MMWR 1992), and a national task force was convened to discuss the benefits and risks of fortifying the food supply with folate (Federal Register 1993). Based on recommendations by this task force, the Food and Drug Administration (FDA) has recently required that cereal grain products be fortified at the level of 140 μg/100 g product (Federal Register 1996).

In light of new evidence that low folate intakes may contribute to risk of atherosclerosis through elevated levels of plasma homocysteine (Selhub et al. 1995), some scientists and policy proponents have recommended folate supplementation for the broader population (Stampfer and Malinow 1995). A debate has ensued about whether supplementation is necessary—and advisable—or whether increased efforts to improve dietary patterns could be effective. Unlike vitamin E, for which the medical benefits presented by most recent scientific research generally require levels beyond those available in usual diets (Rimm et al. 1993), it is possible that folate status, and consequent homocysteine status and its associated risks, may be improved with diet alone. Diet changes to increase folate intake—more fruits, vegetables, and cereals—would have additional benefits, associated with the wide variety of healthful nutrients and non-nutrient factors bal-

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anced in these foods. How great a dietary change is required to obtain 400 μg of folate from diet? What proportion of the population could feasibly make these changes? These remain open questions.

The question of diet improvement vs. supplement or fortification is particularly relevant for one segment of the population, namely, the elderly. A traditional concern has been that high levels of folate intake might correct the hematologic manifestations of vitamin B-12 deficiency, a common condition in the elderly, and thereby contribute to delayed diagnosis and deterioration in nerve function. On the other hand, low folate status is also a common problem among the elderly (Rosenberg et al. 1982, Selhub et al. 1993), and recent data suggest that even low normal levels may be associated with elevated homocysteine, a risk factor for vascular disease in this population (Selhub et al. 1995). To evaluate the potential effects associated with change in diet or supplement use, we have to understand the existing patterns of folate intake and status among the elderly. In this paper, we explore the patterns of folate intake in the diet of elderly participants in the Framingham Heart Study and the relationship between intake and concentrations of plasma folate and plasma homocysteine in a population at considerable risk of vascular disease.

SUBJECTS AND METHODS

The Framingham Heart Study, which began in 1948–50, is a population-based longitudinal study of heart disease risk factors (Dawber et al. 1957). Subjects have been examined every 2 y since the beginning of the study. The initial cohort included 5209 men and women aged 30–62 y. During the 20th cycle of data collection, conducted in 1988–89, more than 1200 subjects were still living.

Prior to this 20th cycle examination, the Willett 126 item semi-quantitative food-frequency questionnaire (Willett et al. 1985) was mailed to members of the cohort for them to complete and bring to their examination. This dietary instrument has been validated for several nutrients, including folate (Jacques et al. 1993) and has been shown to rank individuals very well in relation to their actual intake. The energy-adjusted correlation between folate intake and plasma folate concentration in a group of 139 men and women aged 40–83 years, was 0.63, the highest of the 12 relationships of micronutrient intake to biochemical status tested in that study (Jacques et al. 1993). Despite its semi-quantitative nature, calculated mean nutrient intakes using this method have been shown to resemble closely those obtained by diet records for several nutrients (Willett et al. 1987). A comparison of total folate intake and plasma homocysteine in this population has been reported previously by Selhub et al. (1993); it showed significantly lower homocysteine levels among those with higher folate intakes. One thousand sixty-eight forms were completed. We excluded individuals who reported intakes of <2.5 MJ (600 kcal) or >16.7 MJ (4000 kcal) and any individuals who had data missing for more than 12 food items. An additional 89 subjects had no blood measurements, resulting in 885 available subjects for this analysis.

Blood was drawn from subjects during the cycle 20 examination visit and stored at −80°C. Plasma folate levels were determined, using a microbial assay described by Tamura et al. (1990). A subset of 385 of the subjects as also evaluated for plasma folate concentrations using the Ciba-Corning radioassay kit (Medfield, MA). The correlation between the two methods was 0.38; correlations between each method and dietary folate intake were 0.56 for the microbial assay and 0.43 for the radioassay (Selhub et al. 1993). All results presented here are based on data from the microbial assay. Homocysteine levels were measured using the method of Araki and Sako (1987). The protocol and use of blood samples were approved by the Human Investigation Review Board at Tufts University.

Mean folate intake, and plasma folate and homocysteine concentrations were determined by gender and age group and were compared for significant differences using the general linear models (GLM) procedure of SAS with log-transformed variables. Gender differences were adjusted for age and vice versa; for folate intake, total energy intake was also adjusted. Dominant sources of folate were identified by defining the contribution to total folate intake made by each food item on the food-frequency questionnaire, including supplements, and ranking them according to a method similar to that used by Subar et al. (1989) for the HANES II study. In addition to contributions to the whole group, we examined these rankings by gender and by age group (67–69; 70–79; and 80–95 y).

Based on the results of these rankings, the top sources of folate were identified for further testing. These included supplements, breakfast cereal and orange juice. When food sources were added together by food group, a fourth major source became clear—green leafy vegetables. In this population, legumes, usually a good source of folate, were not consumed frequently enough to examine further. Users of supplements, and those who consumed each of the major food sources, i.e., breakfast cereals, orange juice and green leafy vegetables (including spinach, kale, iceberg, romaine or leaf lettuce, and broccoli), at least 2 times/wk were compared with those using these items <2 times/wk for folate intake, plasma folate and homocysteine concentrations. We compared unadjusted means with *t* tests, and log-transformed variables adjusted for age, gender, total energy intake, as well as for foods and supplement intake, using the GLM procedure of SAS. These food use items were also examined after adjustment for in-

TABLE 1

Mean folate intake, and plasma folate and homocysteine concentrations by gender and age group in the Framingham Heart Study[1]

(*n*)	Men (340)	Women (545)	67–69 (72)	70–79 (636)	80–95 (177)
Folate intake, *μg/d*	366	389*	401	380	372
Plasma folate, *nmol/L*	12.7	16.3*	14.0	14.7	16.1
Homocysteine, *μmol/L*	13.2	12.1**	11.4	12.1	14.3***

1 Means presented are unadjusted; statistical comparisons were tested with log-transformed variables in the general linear models (GLM) procedure of SAS. Models included gender and age category and, for folate intake only, total energy intake.

* Women > men; $P < 0.05$; **women < men; $P < 0.01$; ***age groups significantly different; $P < 0.0001$.

take of all others by including them jointly in a model with age, gender and total energy intake.

In both the rankings of foods to folate intake and the food use comparisons, breakfast cereals and fruits and vegetables were of central importance. We created an overall variable for frequency of total fruit and vegetable intake by summing the intake of all fruits and vegetables reported on the questionnaire except potatoes and legumes for the following two reasons: *1*) with the exception of orange juice, individual fruits and vegetables are used less regularly and contribute to folate intake in a complementary manner for different individuals; and *2*) increasing total fruit and vegetable intake is a major current nutrition objective. To examine the dose response to intake of these key food groups, we examined folate intake and plasma folate and homocysteine concentrations across quintiles of frequency of intake for *1*) breakfast cereals, and *2*) fruits and vegetables, adjusting for age, sex and total energy intake. Significance tests for trend were assessed with multiple regression analysis.

RESULTS

Unadjusted mean folate intake, and plasma folate and homocysteine concentrations are presented by gender and age group in **Table 1.** After transformation and adjustment for age and total energy intake (the latter for folate intake only), women had significantly higher folate intakes and plasma folate concentrations, and significantly lower homocysteine concentrations. After control for gender, only homocysteine concentrations differed by age group—those greater than 80 years of age had significantly higher homocysteine concentrations without significant differences in plasma folate concentration.

The top 40 foods (or supplements) contributing to total population folate intake are listed in **Table 2,** based on responses to the 126 items on the food-frequency questionnaire and ranked by percentage of contribution of food groups to total population intake. These rankings include consideration of the frequency of consumption and the prevalence of consumption in the population as well as the concentration of folate in each food source. As noted above, the major contributors to folate intake for this group were cold breakfast cereals, multivitamins and orange juice. After these, the percentage contributions were much lower per food item, led by pizza, iceberg lettuce, spinach, white bread, bananas, leaf lettuce, oranges and potatoes.

Rankings were generally similar for men and women, with some exceptions. Multivitamins contributed 14% of folate intake for women, but only 10% for men. Foods which ranked as greater folate contributors for women compared with men included lettuce, oranges, broccoli, cauliflower, winter squash, grapefruit and mixed vegetables; foods which ranked as greater folate contributors for men included bread, beans, eggs, whole milk, nuts, corn and particularly beer, which ranked as the number 10 source for men, compared with number 74 for women.

Older subjects had greater proportions of their folate intake from breakfast cereals: those aged 80–95 y, 17%; 70–79 y, 13%; and 67–69 y, 12%. Other foods ranked as greater folate contributors among the oldest group included bread, potatoes, oranges, liver, fruit jams and jellies, winter squash, carrots and corn. Younger subjects obtained more folate from pizza, lettuce, broccoli, beans, beer, nuts, brussels sprouts, candy bars and mixed vegetables than did older subjects.

Both unadjusted mean folate intake, plasma folate and homocysteine concentrations, and means untransformed from logged values after adjustment for age, gender, total energy intake and (for foods) supplement intake are presented in **Table 3** by supplement and food use. Significance tests and adjusted means, without energy adjustment were also calculated with no meaningful difference in results. Average folate intakes were about twice as high for supplement users compared with non-users. Plasma folate concentrations were much greater among supplement users and homocysteine levels were much lower ($P < 0.0001$).

Among the foods and food groups tested, all were significantly associated with greater folate intake. Use of breakfast cereals at least 2 times/wk (average 6.0 times/wk) was most clearly associated with higher plasma folate ($P < 0.0001$) and lower homocysteine ($P < 0.0001$) concentrations, compared with less frequent or non-users (average 0.4 times/wk). After adjustment, orange juice consumption (average 6.6 vs. 0.3 times/wk) was associated with higher plasma folate, but not with lower homocysteine concentration. Green leafy vegetable use, including broccoli, (average 6.6 vs. 0.9 times/wk) was associated with both higher folate ($P <$

TABLE 2

Major contributors to folate intake in the Framingham Heart Study cohort men and women aged 67–95 y [1]

Rank	Source	% contribution	Men	Women	Subgroup rank Age 67–69	70–79	80–95
1	Cold cereal	13.3	1	2	3	2	1
2	Multivitamins	12.8	3	1	1	1	2
3	Orange juice	12.4	2	3	2	3	3
4	Pizza	3.3	4	5	4	4	10
5	Iceberg lettuce	3.2	7	4	5	5	8
6	Spinach, cooked	2.8	6	6	8	6	5
7	White bread	2.6	5	10	7	9	4
8	Bananas	2.6	8	7	6	7	6
9	Romaine/leaf lettuce	2.4	9	9	9	8	12
10	Oranges	2.3	13	8	16	10	9
11	Potatoes	2.2	11	11	14	11	7
12	Skim milk	2.1	15	12	13	12	13
13	Peas/lima beans	1.9	16	15	12	14	11
14	Broccoli	1.9	18	13	10	13	16
15	Beans/lentils	1.7	12	18	11	16	18
16	Tomatoes	1.7	17	16	18	15	19
17	Eggs	1.6	14	19	15	17	15
18	String beans	1.4	21	17	21	18	17
19	English muffins/bagels	1.2	19	20	19	19	22
20	Liver	1.0	23	21	27	24	14
21	Tomato sauce	1.0	24	22	20	21	25
22	Beer	0.9	10	74	25	20	34
23	Whole milk	0.9	22	29	24	25	20
24	Nuts	0.9	20	26	17	22	28
25	Peanut butter	0.9	25	28	23	23	23
26	Jams/jellies	0.8	27	25	40	28	21
27	Other fish	0.8	29	24	28	26	24
28	Cauliflower	0.7	34	23	26	27	30
29	Winter squash	0.7	35	27	37	29	26
30	Carrots, cooked	0.6	33	30	42	30	27
31	Cantaloupe	0.6	37	31	31	31	35
32	Corn	0.6	28	35	39	32	31
33	Beets	0.6	32	33	36	33	33
34	Meat, main dish	0.6	30	34	35	34	32
35	Pasta	0.5	31	37	34	35	39
36	Grapefruit	0.5	44	32	30	36	38
37	Brussels sprouts	0.5	39	36	32	37	43
38	Candy bars	0.5	36	39	33	38	40
39	Other cheese	0.5	38	42	39	41	37
40	Mixed vegetables	0.4	46	38	41	39	48
	Cumulative total	88%					

[1] Values are means, $n = 885$.

0.05) and lower homocysteine ($P < 0.01$) concentrations.

Because the data in Table 3 do not take into account other food sources of folate in the diet, all of the selected food items were included jointly to examine the effects of inclusion of each item after adjustment for the inclusion of other major folate sources (data not shown). The results of this more adjusted model did not differ greatly from the unadjusted results. Intake from each of the sources examined in Table 3 remained strongly associated with folate intake ($P < 0.0001$) after control for the others. Frequent use of supplements and of breakfast cereals remained strongly ($P < 0.0001$) related to greater plasma folate and lower homocysteine concentrations; green leafy vegetables remained significantly related to these two measures ($P < 0.05$). Orange juice was still associated with greater plasma folate ($P < 0.01$), but not with homocysteine concentration. The age and gender differences presented in Table 1 also remained significant after adjustment for use of these specific food sources of folate.

Folate and homocysteine concentrations followed a clear and significant dose response with frequency of intake of breakfast cereals, and of fruits and vegetables, respectively, after adjustment for age, gender, total energy intake and use of folate supplements (**Tables 4 and 5**). More frequent use of cereals was associated with higher plasma folate concentrations ($P < 0.0001$), rang-

TABLE 3

Folate intake and plasma folate and homocysteine concentrations by supplement and food use in the Framingham Heart Study

	Folate intake		Plasma folate		Homocysteine	
Use?	no	yes	no	yes	no	yes
	µg/d		*nmol/L*		*µmol/L*	
Supplement with folate	302	621***	10.6	28.3***	13.3	10.1***
Adjusted[1]	276	559***	8.1	22.2***	12.3	9.6***
(*n*)	(667)	(218)				
Breakfast cereal ≥ 2 times/wk	285	431***	12.4	15.5**	13.6	11.9***
Adjusted	263	369***	8.1	11.9***	12.5	11.1***
(*n*)	(305)	(580)				
Orange juice ≥ 2 times/wk	328	408***	14.9	15.0	12.7	12.4
Adjusted	285	354***	9.5	10.9**	11.6	11.5
(*n*)	(306)	(579)				
Green leafy vegetables[2] ≥ 2 times/wk	304	410***	14.7	15.2	13.9	12.1**
Adjusted	270	355***	9.5	10.8*	12.3	11.4**
(*n*)	(222)	(640)				

[1] Adjusted for age, gender, total energy intake and for foods, supplement intake; dependent variables were log transformed; presented means are untransformed from the log scale.

[2] Spinach, kale, iceberg, romaine or leaf lettuce, or broccoli.

$*P < 0.05$; $**P < 0.01$; $***P < 0.0001$.

ing from 7.9 nmol/L at intakes of 0–0.5 servings/wk to 13.5 nmol/L when cereal was consumed at least daily. The association between cereal intake and homocysteine level also showed a strong dose response at lower levels of intake, but appeared to plateau at about 5–6 servings/wk.

Fruit and vegetable intake (**Table 5**) showed a similar pattern. Folate levels ranged from 8.6 nmol/L for the lowest intake quintile (0–2.9 servings/d) to a plateau with a maximum mean value of 12.5 nmol/L for ~6 servings/d at the 4th quintile. Homocysteine concentration followed a parallel pattern, also reaching a low plateau at about 5–6 reported servings per day.

DISCUSSION

Unlike some nutrients for which variation in absorption and homeostatic mechanisms make blood levels a poor marker of dietary intake, statistical comparison of total folate intake to plasma folate concentrations reveals a strong relationship (Jacques et al. 1993, Selhub et al. 1993). Those with higher intakes tended to have higher folate status as assessed by plasma levels. Given this relationship, it is important to know how food choices among the elderly affect folate and homocysteine status. These analyses show that food choice is indeed clearly associated with folate and homocysteine status.

The major contributors to folate intake in this population of elders were cold breakfast cereals, multivitamins and orange juice. Other individual fruits and vegetables contributed much smaller but important amounts, which, when added together, were the dominant source of total folate intake in the diet. Women tended to have higher folate intakes and obtained more from supplements and from fruits and vegetables than men; men consumed more from beer, bread and eggs.

TABLE 4

Folate and homocysteine status by quintile of average weekly frequency of breakfast cereal intake in the Framingham Heart Study[1]

Frequency mean	Quintile (range)	Folate intake	Plasma folate	Homocysteine
		µg/d (CI)	*nmol/L (CI)*	*µmol/L (CI)*
0.1	(0–0.5)	257 (244–270)	7.9 (7.2–8.7)	12.4 (11.8–13.0)
2.1	(0.9–3.0)	309 (294–325)	9.2 (8.4–10.1)	12.0 (11.4–12.6)
4.6	(3.5–5.5)	350 (328–374)	11.3 (10.0–12.8)	11.3 (10.6–12.0)
6.6	(6.0–7.0)	384 (367–404)	12.8 (11.7–14.0)	10.8 (10.3–11.3)
10.2	(7.5–42.5)	414 (385–444)	13.5 (11.8–15.4)	11.0 (10.3–11.8)
P for trend		0.0001	0.0001	0.0001

[1] Data are means (95% confidence interval). Analysis was performed on log-transformed variables; data presented are adjusted for age, gender, total energy intake and folate supplement intake and are untransformed from the log scale.

TABLE 5

Folate and homocysteine status by quintile of average daily frequency of fruit and vegetable intake in the Framingham Heart Study[1]

Frequency mean	Quintile (range)	Folate intake	Plasma folate	Homocysteine
		μg/d	*nmol/L*	*μmol/L*
1) 2.0	(0–2.9)	234 (221–247)	8.6 (7.6–9.5)	12.1 (11.4–12.8)
2) 3.5	(2.9–4.0)	303 (287–319)	9.4 (8.5–10.4)	11.9 (11.3–12.6)
3) 4.6	(4.0–5.2)	348 (331–367)	10.1 (9.1–11.1)	11.6 (11.0–12.2)
4) 5.9	(5.2–6.7)	364 (345–384)	12.5 (11.3–13.9)	11.1 (10.5–11.7)
5) 8.9	(6.7–18.8)	430 (405–455)	12.0 (10.7–13.4)	11.0 (10.4–11.7)
P for trend		0.0001	0.001	0.005

[1] Data are means (95% confidence interval). Analysis was performed on log-transformed variables; data presented are adjusted for age, gender, total energy intake and folate supplement intake and are untransformed from the log scale.

Those above 80 y of age consumed more from cereal, bread and potatoes, whereas younger subjects consumed more from pizza, lettuce, broccoli, beans and nuts.

Twenty-five percent of subjects were consuming vitamin supplements, and this group clearly had the highest folate intakes and blood levels and lowest homocysteine levels. Use of selected food types was also related (although not as strongly) not only to folate intake, but also to plasma folate and homocysteine concentrations. The significant relationship between inclusion of breakfast cereals and plasma folate and homocysteine concentrations is likely to be due largely to folic acid fortification of cereals. Examination of these concentrations by quintile of cereal use reveals very strong relationships which tend to plateau at about 5–6 servings/wk. The strength of these relationships provides a strong argument for the value of including cereal in a regular breakfast schedule.

Although use of orange juice was associated with much greater folate intake, its association with plasma folate was less significant and, surprisingly, was not significantly related to homocysteine concentration in this sample. Inclusion of leafy green vegetables, including broccoli, in the diet was related to significantly greater folate and lower homocysteine concentrations, after adjustment for other variables. The relationship of intake of these vegetables to homocysteine concentration appeared to be stronger than the relationship with plasma folate concentration.

In addition to the major contributors—orange juice and green leafy vegetables—a variety of fruits and vegetables contributed small amounts of folate to the total group intake, and differing foods may be equally important to the intakes of different individuals. Examination of total fruit and vegetable intake by quintile, therefore provides more power to detect the effect of overall fruit and vegetable intake. This information is also important because current public health recommendations include an emphasis on increasing total fruit and vegetable intake from a variety of sources. Taken together, greater frequency of consumption of fruits and vegetables had a clear and dramatic relationship with folate intake, and with plasma folate and homocysteine concentrations. Interestingly, plasma folate and homocysteine concentrations appeared to plateau at just over five servings per day, providing yet another reason to support interventions associated with the National Cancer Institute's "Five a day" campaign.

Recent studies linking homocysteine concentration with vascular disease underscore the likely benefits of reducing these concentrations (Kang et al. 1992, Selhub et al. 1995, Stampfer et al. 1992, Ueland et al. 1992). Our results clearly suggest that diet choices are associated with folate status and homocysteine concentration. In this group of elderly subjects, the lowest concentrations of homocysteine were seen with multivitamin use; however, nearly comparable levels were also seen among frequent consumers of breakfast cereal and of fruits and vegetables. Another recent study, using dietary data collected from men in this Framingham cohort in 1966 through 1969, found that over the course of 20 y of follow-up, age adjusted risk of stroke decreased across increasing quintile of fruit and vegetable intake, in a pattern similar to our current finding of increased homocysteine concentration with increasing quintile of fruit and vegetable intake (Gillman et al. 1995). As evidence for the importance of folate status to health accumulates, along with evidence of substandard folate status among the U.S. population, education to improve folate intake through increased consumption of fruits, vegetables and cereals is important. Our results suggest that greater intake of these foods could increase folate and lower homocysteine concentrations.

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