

Frequent consumption of milk, yogurt, cold breakfast cereals, peppers, and cruciferous vegetables and intakes of dietary folate and riboflavin but not vitamins B-12 and B-6 are inversely associated with serum total homocysteine concentrations in the US population^{1,2}

Vijay Ganji and Mohammad R Kafai

ABSTRACT

Background: Elevated circulating total homocysteine (tHcy) is an independent risk factor for vascular diseases.

Objective: We investigated the relation between dietary intakes and serum tHcy in the US population.

Design: Data from the third National Health and Nutrition Examination Survey (1988–1994) were used to investigate the associations between food consumption frequency and dietary B vitamin intakes and serum tHcy in 5996 persons.

Results: Multivariate-adjusted tHcy concentrations were $\approx 15.2\%$ higher in subjects who never consumed milk than in those who consumed milk >30 times/mo, $\approx 6.4\%$ higher in subjects who never consumed yogurt than in those who consumed yogurt >15 times/mo, $\approx 7.4\%$ higher in subjects who never consumed cold breakfast cereals than in those who consumed cold breakfast cereals >30 times/mo, $\approx 6.3\%$ higher in subjects who never consumed peppers (includes red, yellow, green, and hot chili peppers) than in those who consumed peppers >30 times/mo, and $\approx 16.5\%$ higher in subjects who never consumed cruciferous vegetables than in those who consumed cruciferous vegetables >30 times/mo. Consumption of citrus fruit and juices, cheese, meats, coffee, or tea had no significant association with tHcy. Folate ($\beta = -0.0017$, P for trend = 0.004) and riboflavin ($\beta = -0.2851$, P for trend = 0.027), but not vitamin B-6 ($\beta = 0.0505$, P for trend = 0.70) and cobalamin ($\beta = -0.0035$, P for trend = 0.58), were inversely related to serum tHcy after adjustment for confounders.

Conclusions: In this population-based study, milk, yogurt, cold breakfast cereals, peppers, and cruciferous vegetables were inversely related to serum tHcy. This association may be explained by increased intakes of folate and riboflavin. *Am J Clin Nutr* 2004; 80:1500–7.

KEY WORDS Homocysteine, third National Health and Nutrition Examination Survey, NHANES III, heart disease, food-frequency questionnaire, dietary recall, B vitamins, folic acid, riboflavin, milk, yogurt, breakfast cereal, fruit, vegetables, meat, coffee

INTRODUCTION

Homocysteine (Hcy) is a nonprotein-forming amino acid formed from methionine in the demethylation reaction. Several

studies have shown that elevated total homocysteine (tHcy) concentrations in blood is associated with an increased risk of cardiovascular diseases (1) and decreased cognitive function (2). A 10% increase in circulating Hcy increases the risk of heart diseases by 10–15% (3). The mechanisms that may explain the association between tHcy and heart disease include toxic effects on endothelial cells, increased smooth cell proliferation, LDL oxidation, and thrombus formation (4–6).

Hcy metabolism is regulated by various enzymes and B vitamins. Methylene tetrahydrofolate reductase, a flavin adenine dinucleotide (coenzyme of riboflavin) dependent enzyme, catalyzes N^5 - N^{10} methylene tetrahydrofolate to N^5 -methyl tetrahydrofolate. N^5 -Methyl tetrahydrofolate is required for remethylation of Hcy to methionine by a vitamin B-12–dependent enzyme, methionine synthase (7). Flavin mononucleotide, a riboflavin coenzyme, is required for B-6 phosphate oxidase, which converts vitamin B-6 to its coenzyme form, pyridoxal phosphate (PLP) (8). PLP-dependent enzymes such as cystathionine- β -synthase and γ -cystathionase are needed for transsulfuration of Hcy to cysteine (9). Genetic abnormalities in enzymes (10–12) and deficiencies of B vitamins (13–15) involved in methionine metabolism lead to elevated tHcy. Low blood concentrations of folate and vitamins B-12 and B-6 are associated with elevated circulating tHcy (12, 16, 17). The association between circulating riboflavin concentration and serum tHcy has received little attention.

Despite the clear association between B vitamins and circulating tHcy concentrations, the relation between diet and tHcy is poorly understood. Most studies have focused on the association between individual nutrients, such as folate and tHcy (16, 18–20). A few studies reported an inverse relation between vegetable and fruit consumption and circulating tHcy concentrations (21–24). No information is available regarding the relation between

¹ From the Department of Clinical Nutrition, College of Health Sciences, Rush University Medical Center, Chicago (VG), and the Department of Mathematics, San Francisco State University, San Francisco (MRK).

² Reprints not available. Address correspondence to V Ganji, Department of Clinical Nutrition, 1700 West Van Buren Avenue, Suite 425, Rush University Medical Center, Chicago, IL 60612. E-mail: vijay_ganji@rush.edu. Received February 25, 2004.

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tHcy concentration and intake of a specific type of vegetable. Very little is reported on the relation between tHcy and intakes of protein-rich foods, riboflavin, and vitamin B-6. The relation between food patterns and intakes of B vitamins and circulating tHcy has not been studied in a nationally representative sample. Therefore, we investigated the associations between frequency of consumption of various foods and dietary intakes of folate, riboflavin, vitamin B-12, and vitamin B-6 and serum tHcy concentration in a nationally representative sample survey of US residents.

SUBJECTS AND METHODS

Survey design and sample

The third National Health and Examination Survey (NHANES III) is a complex, stratified, probability sample survey of noninstitutionalized US residents aged ≥ 2 mo in 2 phases from 1988 to 1994. The first phase of the survey was conducted in 1988–1991 at 44 locations; the second phase of the survey was conducted in 1991–1994 at 45 locations. In NAHNES III, young children, older persons, non-Hispanic blacks, and Mexican Americans were oversampled. The data used in this study were derived from the databases released for public use by the National Technical Information Service, Springfield, VA (25–27). The detailed description of the survey methods and analytic guidelines were reported elsewhere (28). The survey included 39 695 subjects. Of this number, 33 994 subjects were interviewed in their homes; 30 818 were examined in mobile clinics and 493 in their homes. NHANES III included 1614 subjects with diabetes, 338 pregnant women, and 100 lactating women. Serum tHcy concentrations were measured in 8585 participants aged >12 y. Therefore, the subjects for this study were drawn from this sample. Participants with diabetes, who were pregnant or lactating, or who had missing or incomplete data for dietary intakes and confounding variables were excluded. Also, we excluded participants aged ≥ 90 y because all participants aged >89 y were reported as being 90 y of age to protect the confidentiality of these persons. This allowed us to use age as a continuous variable in the analysis. After the aforementioned criteria were applied, the current study was based on a total of 5996 subjects (2753 men and 3243 women; weighted sample size = 61 934 853).

Measurements

Depending on the age of the participant, data were collected on demographics, socioeconomic status, physical and health conditions, lifestyle behaviors, biochemical measurements of blood and urine, anthropometric measurements, and dietary intakes. Blood was collected by venipuncture in the mobile clinics according to standard protocol (29). Of the 5996 participants, 3523 ($\approx 59\%$) subjects fasted for ≥ 9 h, 1987 ($\approx 33\%$) subjects fasted for 5 to <9 h, and 486 ($\approx 8\%$) subjects fasted for <5 h. The duration of fasting had no measurable effect on serum tHcy (30). Serum was separated by centrifugation ($1115 \times g$ for 15 min at room temperature) after blood samples were held at room temperature for 30–60 min. Sera were frozen at -20°C and transported on dry ice to the Centers for Disease Control and Prevention for priority analysis. After priority analysis, surplus sera were stored at -70°C for 8 mo to 3 y. Serum tHcy concentrations were measured in these surplus sera in phase 2 of NAHANES III

(1991–1994) at the US Department of Agriculture Human Nutrition Research Center on Aging after approval by the New England Medical Center Human Investigations Review Committee. Serum tHcy concentrations were measured with the HPLC method (31).

Dietary assessment

Food intake data were obtained through administration of an 80-item qualitative food-frequency questionnaire (FFQ) to survey participants. Food-frequency data were collected as part of the household interview. Depending on the respondent's language preference, the FFQ was administered either in English or in Spanish by trained interviewers. Before intake collection, the FFQ was pretested and modified to be culturally appropriate for non-Hispanic whites, non-Hispanic blacks, and Mexican Americans. Participants were asked how often they ate or drank a particular food or drink over the past month. Food intakes were reported as the number of times consumed per day, per week, or per month or never. Food-frequency-consumption data were standardized as times per month with the use of the conversion factors 4.3 wk/mo and 30.4 d/mo rounded to the nearest whole number. For this study purpose, reported foods were categorized into 25 food groups. These food groups were milk (milk to drink or on cereal), yogurt (including frozen yogurt), cheeses (all types), meats (beef, pork, poultry, and organ and processed meats), seafood (fish and shellfish), eggs, cold and hot breakfast cereals, rice, pasta, breads (all breads, rolls, bagels, muffins, crackers, and tortillas), citrus (oranges, grapefruit, and tangerines) and noncitrus fruit and juices, starches (all potatoes, yams, and squash), peppers (green, red, yellow, and hot chili peppers), cruciferous (broccoli, Brussels sprouts, and cauliflower) and other vegetables, legumes (beans, lentils, peanuts, and garbanzo, black, and pinto beans), cola beverages (both diet and regular), fruit-flavored beverages, coffee, tea, desserts (cakes, cookies, pies, pastries, chocolate candies, etc), ice creams (including ice milk and milk shakes), and fats (margarine, butter, oil and vinegar, mayonnaise, and salad dressings).

In addition to FFQ, dietary intakes were collected through administration of 24-h food recalls. Food-recall data were collected by using an automated, microcomputer-based dietary interview and coding system known as Dietary Data Collection. Dietary interviews were conducted in English and Spanish, depending on the participant's preference, in a private room to ensure confidentiality. Participants reported all food and beverages consumed, except for plain water, for the previous 24-h time period (midnight to midnight). The nutrient intakes did not include nutrients from supplements, antacids, medications, and salt and seasonings added to prepared foods at the table. The nutrient composition of the foods reported on the food recalls was based on the US Department of Agriculture Survey Nutrient Databases (32). These databases contained food-composition values that were appropriate for the time period during which the data were collected. Many quality-control measures were used to ensure the accuracy of the food recalls. A detailed description of the dietary intake methods was published elsewhere (28). In the current study, we analyzed the relation between serum tHcy concentration and nutrient intake values for folic acid, riboflavin, and vitamins B-12 and B-6 because these vitamins are involved in the regulation of Hcy metabolism.

Statistical analyses

To account for the complex survey design, we used SUDAAN statistical software (SUDAAN for WINDOWS, version 8.0.2; Research Triangle Institute, Research Triangle Park, NC). Means and SEs were estimated by using sample weights. Sample weights incorporate the differential probabilities of selection and include adjustments for noncoverage and nonresponse bias. A detailed description of the nonresponse adjustment and survey coverage was published elsewhere (28). SAS (SAS for WINDOWS, version 8.0; SAS Institute Inc, Cary, NC) in conjunction with SUDAAN was used to analyze the data files.

Because intakes of various foods or dietary nutrients might be correlated with each other, and therefore might potentially confound each other in the analysis, we tested for colinearity with the use of multivariate models. We found no colinearity among the food groups or among the B vitamins under consideration. After removing the nonsignificant food groups and dietary B vitamins in a stepwise fashion from the multivariate models, we calculated variance inflation factors. The variance inflation factors for all of the food groups ranged from 1.08 for yogurt to 1.18 for peppers; the values were 2.36 and 2.56 for folic acid and riboflavin, respectively. Colinearity is a concern if the variance inflation factor is >10 (33).

Differences in the frequency of food consumption and intakes of B vitamins between males and females were assessed with a two-tailed *t* test. The frequency of consumption of food or food groups was classified into 4 categories, ie, 0, 1–15, 16–30, and >30 times/mo. Dietary intakes of folic acid, riboflavin, and vitamins B-12 and B-6 were categorized into quartiles. Regression models adjusted for age and sex (male and female) and for multivariates [age, sex (male and female), race-ethnicity (non-Hispanic white, non-Hispanic black, and Mexican American), vitamin and mineral supplement use (yes or no), alcohol consumption (alcohol intake was calculated by combining intake of beers, wines, and hard liquors), serum cotinine (a measure of smoking), body mass index (weight in kg/ht in m^2), and serum creatinine] were used to determine the effect of each food or food group variable or intake of B vitamin on serum tHcy.

We used 2 separate multivariate models; one was based on frequency of food consumption data and the other on dietary intakes of B vitamins. Sex- and age-adjusted and multivariate-adjusted means and SEs were generated with analysis of covariance. Mean comparisons were performed with the Tukey-Cramer multiple-comparison test among categories within a food group or dietary B vitamin intake variable if that variable was found significant in the analysis of covariance for the Wald *F* statistic. Additionally, age- and sex-adjusted and multivariate-adjusted regression analyses were performed for serum tHcy concentration as a dependant variable with food-frequency data (times/mo) or intakes of B vitamins as independent continuous variables. In all analyses, a $P < 0.05$ was considered statistically significant.

RESULTS

Of the 5996 subjects, $\approx 46\%$ were males ($n = 2753$), $\approx 54\%$ were females ($n = 3243$), $\approx 39\%$ were non-Hispanic whites ($n = 2308$), $\approx 32\%$ were non-Hispanic blacks ($n = 1951$), and $\approx 29\%$ were Mexican Americans ($n = 1737$). Data on the subjects' characteristics are given in **Table 1**. The age of the female subjects was slightly but significantly higher than the age of the male

TABLE 1

Characteristics of the study population by sex in the third National Health and Nutrition Examination Survey¹

Characteristic	Male ($n = 2753$)	Female ($n = 3243$)
Race-ethnicity (n)		
Non-Hispanic white	972	1336
Non-Hispanic black	859	1092
Mexican American	922	815
Vitamin and mineral supplement use (n)		
Yes	907	1364
No	1846	1879
Age (y)	$38.4 \pm 0.4^{2,3}$	41.1 ± 0.4
BMI (kg/m^2)	26.3 ± 0.2	25.8 ± 0.2
Serum cotinine ($\mu mol/L$)	2.5 ± 0.3^3	0.9 ± 0.1
Serum creatinine ($\mu mol/L$)	103.2 ± 0.4^3	85.7 ± 0.3
Alcohol intake (times/mo)	10.2 ± 0.5^3	6.5 ± 0.3
Frequency of food consumption (times/mo)		
Milk	15.9 ± 0.7^3	14.7 ± 0.5
Yogurt	4.0 ± 0.3^3	4.4 ± 0.2
Cheeses	9.7 ± 0.3^3	8.4 ± 0.2
Meats	29.7 ± 0.6^3	24.4 ± 0.5
Seafood	4.6 ± 0.1^3	4.3 ± 0.1
Eggs	5.1 ± 0.2^3	4.1 ± 0.1
Cold breakfast cereals	8.7 ± 0.3	8.6 ± 0.3
Hot breakfast cereals	4.1 ± 0.2^3	4.5 ± 0.2
Rice	4.6 ± 0.2	4.6 ± 0.1
Pasta	2.9 ± 0.1	2.9 ± 0.1
Breads	32.4 ± 0.8^3	30.4 ± 0.6
Citrus fruit and juices	12.4 ± 0.5^3	14.0 ± 0.5
Noncitrus fruit and juices	15.6 ± 0.6^3	20.3 ± 0.6
Starches	11.4 ± 0.3	10.7 ± 0.2
Peppers ⁴	5.7 ± 0.2^3	5.0 ± 0.2
Cruciferous vegetables	6.2 ± 0.2^3	7.7 ± 0.2
Other vegetables	32.3 ± 0.8	38.4 ± 0.8
Legumes	8.4 ± 0.3^3	6.8 ± 0.2
Cola beverages	20.3 ± 0.8^3	16.5 ± 0.6
Fruit-flavored beverages	8.2 ± 0.5^3	8.7 ± 0.5
Coffee	32.6 ± 1.6^3	31.0 ± 1.3
Tea	11.5 ± 0.7	12.3 ± 0.7
Desserts	11.2 ± 0.4	10.3 ± 0.3
Ice cream	4.4 ± 0.2^3	3.7 ± 0.1
Fats	25.7 ± 0.8	27.3 ± 0.6
Dietary intakes of B vitamins		
Folic acid (μg)	274.2 ± 6.0^3	191.0 ± 3.8
Vitamin B-12 (μg)	4.6 ± 0.13^3	2.6 ± 0.07
Vitamin B-6 (mg)	2.0 ± 0.04^3	1.3 ± 0.02
Riboflavin (mg)	2.2 ± 0.04^3	1.4 ± 0.02

¹ $n = 5996$; weighted sample size (n) = 61 934 853.

² Geometric $\bar{x} \pm SE$ (all such values).

³ Significantly different from females, $P < 0.05$ (two-tailed *t* test).

⁴ Includes green, red, yellow, and hot chili peppers.

subjects ($P < 0.001$). Males and females did not differ significantly in BMI. Serum cotinine and creatinine and alcohol intake were significantly higher in males than in females. Men and women differed significantly in the frequency of consumption of certain foods. For example, men consumed milk, cheese, meats, seafood, eggs, breads, peppers, legumes, cola beverages, coffee, and ice cream more frequently than did women; women consumed yogurt, hot breakfast cereals, citrus and noncitrus fruit and juices, cruciferous vegetables, and fruit-flavored beverages more frequently than did men. Dietary intakes of folic acid, riboflavin,

and vitamins B-12 and B-6 were significantly higher in males than in females ($P < 0.0001$).

The association between frequency of consumption of various food and food groups and serum tHcy is presented in **Table 2**. In the multivariate-adjusted regression analysis, the frequency of consumption of milk ($\beta = -0.0129$, P for linear trend = 0.0029), yogurt ($\beta = -0.0238$, P for linear trend = 0.0218), cold breakfast cereals ($\beta = 0.0411$, P for linear trend < 0.0001), peppers ($\beta = -0.0175$, P for linear trend = 0.0208), and cruciferous vegetables ($\beta = -0.0212$, P for linear trend = 0.0058) was inversely associated with serum tHcy.

Multivariate-adjusted serum tHcy concentrations were $\approx 15.2\%$ higher in subjects who never consumed milk than in those who consumed milk >30 times/mo, $\approx 6.4\%$ higher in subjects who never consumed yogurt than in those who consumed yogurt >15 times/mo, $\approx 7.4\%$ higher in subjects who never consumed cold breakfast cereals than in those who consumed cold breakfast cereals >30 times/mo, $\approx 6.3\%$ higher in subjects who never consumed peppers than in those who consumed peppers >30 times/mo, and $\approx 16.5\%$ higher in subjects who never consumed cruciferous vegetables than in those who consumed cruciferous vegetables >30 times/mo.

In the age- and sex-adjusted regression analysis, cola beverage consumption was positively associated with serum tHcy ($\beta = 0.0088$, P for linear trend = 0.033). However, this association disappeared when the model adjusted for confounding variables in the multivariate regression analysis. The association between citrus fruit and juices and serum tHcy was not significant in the multivariate analyses. When citrus and noncitrus fruit were tested as one combined variable, the association with serum tHcy remained null (data not shown). Also, there was no association between serum tHcy and frequency of consumption of cheeses, seafood, eggs, hot breakfast cereals, rice, pasta, breads, starches, other vegetables (nonpepper and noncruciferous), legumes, fruit-flavored beverages, tea, desserts, ice cream, and fats (data not shown).

The associations between serum tHcy and dietary intakes of folic acid, riboflavin, and vitamins B-12 and B-6 are presented in **Table 3**. We observed an inverse association between folic acid (multivariate-adjusted $\beta = -0.0017$, P for linear trend = 0.0044) and riboflavin (multivariate-adjusted $\beta = -0.2851$, P for linear trend = 0.0272) and serum tHcy. Multivariate-adjusted serum tHcy concentrations were $\approx 12.0\%$ lower in the 4th quartile than in the 1st quartile of folic acid intake and $\approx 8.3\%$ lower in the 4th quartile than in the 1st quartile of riboflavin intake. In the age- and sex- and multivariate-adjusted analyses, no association was observed between serum tHcy and intakes of vitamins B-12 and B-6 in both categorical and continuous forms.

DISCUSSION

In this cross-sectional study, we found that milk, yogurt, cold breakfast cereals, peppers, and cruciferous vegetables were significant determinants of serum tHcy after various potential confounding variables were taken into consideration. To our knowledge, this is the first study that reports an inverse association between serum tHcy and the consumption of dairy products, peppers, and cruciferous vegetables. In addition, intakes of folic acid and riboflavin but not of vitamins B-12 and B-6 were significant determinants of serum tHcy concentrations.

Among all food groups examined, cold breakfast cereals showed the strongest association with serum tHcy (multivariate adjusted $\beta = -0.0411$; P for linear trend < 0.0001). In a separate analysis, when adjusted for dietary folate, the inverse association between cold breakfast cereal and tHcy was no longer present ($P = 0.1810$). This suggests that dietary folate is driving the association between cold breakfast cereals and tHcy. Our observations support the findings by Malinow et al (23), who reported that the daily consumption of breakfast cereal containing 499 and 665 μg folic acid/serving reduced tHcy concentrations by 11% and 14%, respectively. Fortified breakfast cereal is a major contributor of dietary folate in the United States (21). Consumption of fortified breakfast cereal increased dietary folate by $\approx 298 \mu\text{g}$ and serum folate by $\approx 21 \text{ nmol/L}$ (34). Decreased serum tHcy with fortified cereal consumption can mainly be attributable to the highly bioavailable folic acid in the breakfast cereal. Breakfast cereals are fortified with oxidized monoglutamyl folate, which has a higher bioavailability than does the naturally occurring reduced polyglutamyl folate in foods (35).

In this study, subjects who never consumed milk had serum tHcy concentrations that were $\approx 1.4 \mu\text{mol/L}$ higher than those of subjects who consumed milk ≥ 1 time/d. Because people often consume cold breakfast cereal with milk, we specifically tested the colinearity between these 2 foods. We found no colinearity between milk and cold breakfast cereal (variance inflation factor = 1.1). Thus, the inverse association between milk and serum tHcy was not confounded by the usage pattern of cold breakfast cereal. Milk is known for its high content of riboflavin ($\approx 0.4 \text{ mg/cup}$, or 244 g) (36). Milk is a major contributor of riboflavin in the Western diet (37). As discussed earlier, riboflavin coenzymes are needed for both the remethylation and trans-sulfuration of Hcy. Although the role for riboflavin in Hcy metabolism is recognized, the association between dietary riboflavin and tHcy has received little attention. However, there is support for our findings. In the Framingham Study population, Jacques et al (16) reported an inverse association between dietary riboflavin and tHcy ($P < 0.003$). Recently, Moat et al (38) reported an inverse relation between riboflavin status and tHcy. Considering the inverse association between dietary riboflavin and tHcy and the lack of colinearity between milk and cold breakfast cereal, we suspected that the inverse association of tHcy with milk was likely due to its riboflavin content. This assumption is further strengthened by the fact that we found no association between cheese consumption and serum tHcy. Although cheese is a good source of several nutrients, it is a poor source of riboflavin because this water-soluble vitamin is largely lost in whey during cheese preparation.

Despite the fact that citrus fruit and juices are good sources of folate, we found no association between these foods and serum tHcy. Earlier, Tucker et al (21) reported no association between tHcy and orange juice despite a much greater folate intake with orange juice. In their study, folate intake was 354 μg in persons who consumed orange juice 6.6 times/wk compared with 285 μg in those who consumed orange juice 0.3 times/wk. This difference in folate intake resulted a change of only $\approx 1.4 \text{ nmol/L}$ in serum folate with no significant change in tHcy. In our study, the average frequency of consumption of citrus fruit and juices was <0.5 time/d (geometric $\bar{x} = 13.2$ times/mo). It is likely that the lack of association was due to low intakes of citrus fruit and juices, which led to lower dietary folate intakes and insufficient increases in plasma folate, and thus no association with tHcy.

TABLE 2

Serum total homocysteine (tHcy) concentrations by the frequency of consumption of various foods and food groups in the third National Health and Nutrition Examination Survey¹

Food and food group ¹	Sex- and age-adjusted analysis ²			Multivariate-adjusted analysis ^{2,3}		
	Serum tHcy ⁴ <i>μmol/L</i>	$\beta \pm SE^5$	<i>P</i> for β^6	Serum tHcy ⁴ <i>μmol/L</i>	$\beta \pm SE^5$	<i>P</i> for β^6
Milk						
0 times/mo (<i>n</i> = 1066)	10.5 ± 0.4 ^a	-0.0152 ± 0.004	0.0003	10.6 ± 0.4 ^a	-0.0129 ± 0.004	0.0029
1–15 times/mo (<i>n</i> = 2390)	10.2 ± 0.2 ^a			10.2 ± 0.2 ^a		
16–30 times/mo (<i>n</i> = 2039)	9.5 ± 0.2 ^b			9.5 ± 0.1 ^b		
>30 times/mo (<i>n</i> = 501)	9.1 ± 0.2 ^b			9.2 ± 0.2 ^b		
Yogurt						
0 times/mo (<i>n</i> = 4148)	10.0 ± 0.1 ^a	-0.0374 ± 0.011	0.0004	10.0 ± 0.1	-0.0238 ± 0.010	0.0218
1–15 times/mo (<i>n</i> = 1627)	9.7 ± 0.2 ^{a,b}			9.7 ± 0.2		
>15 times/mo (<i>n</i> = 221) ⁷	9.2 ± 0.3 ^b			9.4 ± 0.3		
Meats						
0–15 times/mo (<i>n</i> = 977) ⁷	10.0 ± 0.3	0.0017 ± 0.006	0.7638	10.1 ± 0.3	-0.0036 ± 0.006	0.5198
16–30 times/mo (<i>n</i> = 2156)	9.8 ± 0.2			9.9 ± 0.2		
>30 times/mo (<i>n</i> = 2863)	9.8 ± 0.2			9.8 ± 0.2		
Cold breakfast cereals						
0 times/mo (<i>n</i> = 1780)	10.3 ± 0.2 ^a	-0.0461 ± 0.007	<0.0001	10.2 ± 0.2 ^a	-0.0411 ± 0.007	<0.0001
1–15 times/mo (<i>n</i> = 2964)	10.0 ± 0.1 ^a			9.9 ± 0.1 ^a		
16–30 times/mo (<i>n</i> = 1088)	9.2 ± 0.2 ^b			9.3 ± 0.2 ^b		
>30 times/mo (<i>n</i> = 164)	9.2 ± 0.4 ^{a,b}			9.5 ± 0.4 ^{a,b}		
Citrus fruit and juices						
0 times/mo (<i>n</i> = 710)	9.9 ± 0.4	-0.0037 ± 0.004	0.3693	9.9 ± 0.4	-0.0009 ± 0.004	0.8176
1–15 times/mo (<i>n</i> = 2270)	9.9 ± 0.2			9.9 ± 0.2		
16–30 times/mo (<i>n</i> = 1383)	9.6 ± 0.2			9.7 ± 0.1		
>30 times/mo (<i>n</i> = 1633)	9.9 ± 0.2			10.0 ± 0.2		
Peppers						
0 times/mo (<i>n</i> = 2293)	10.1 ± 0.2 ^a	-0.0197 ± 0.007	0.0053	10.1 ± 0.2	-0.0175 ± 0.008	0.0208
1–15 times/mo (<i>n</i> = 2582)	9.8 ± 0.1 ^{a,b}			9.8 ± 0.1		
16–30 times/mo (<i>n</i> = 651)	9.3 ± 0.2 ^b			9.4 ± 0.2		
>30 times/mo (<i>n</i> = 470)	9.3 ± 0.3 ^{a,b}			9.5 ± 0.3		
Cruciferous vegetables						
0 times/mo (<i>n</i> = 893)	10.6 ± 0.3 ^a	-0.0251 ± 0.008	0.0029	10.6 ± 0.3 ^a	-0.0212 ± 0.008	0.0058
1–15 times/mo (<i>n</i> = 4029)	9.9 ± 0.1 ^b			9.9 ± 0.1 ^b		
16–30 times/mo (<i>n</i> = 834)	9.3 ± 0.2 ^c			9.4 ± 0.2 ^b		
>30 times/mo (<i>n</i> = 240)	9.1 ± 0.4 ^{b,c}			9.1 ± 0.4 ^b		
Cola beverages						
0 times/mo (<i>n</i> = 980)	9.7 ± 0.2	0.0088 ± 0.004	0.0329	9.8 ± 0.3	0.0077 ± 0.004	0.0559
1–15 times/mo (<i>n</i> = 2252)	9.7 ± 0.1			9.7 ± 0.1		
16–30 times/mo (<i>n</i> = 1859)	9.8 ± 0.2			9.8 ± 0.2		
>30 times/mo (<i>n</i> = 905)	10.3 ± 0.2			10.2 ± 0.2		
Coffee						
0 times/mo (<i>n</i> = 2470)	9.8 ± 0.2	-0.0004 ± 0.002	0.8420	9.9 ± 0.2	-0.0007 ± 0.002	0.7210
1–15 times/mo (<i>n</i> = 778)	10.5 ± 0.4			10.4 ± 0.3		
16–30 times/mo (<i>n</i> = 1785)	9.7 ± 0.2			9.6 ± 0.2		
>30 times/mo (<i>n</i> = 963)	9.8 ± 0.2			9.8 ± 0.2		

¹ Subjects (*n* = 5996) were categorized by the frequency of consumption of foods or food groups (times/mo).

² The association between tHcy and the frequency of consumption of cheeses, seafood, eggs, hot breakfast cereals, rice, pasta, breads, starches, legumes, fruit-flavored beverages, tea, desserts, ice cream, and fats was not significant in both analysis of covariance (ANCOVA) and regression models. The noncitrus fruit variable was significant only in the sex- and age-adjusted analysis, and the other vegetable variable was significant only in the multivariate-adjusted ANCOVA (data not shown).

³ Adjusted for age, sex (male and female), race-ethnicity (non-Hispanic white, non-Hispanic black, and Mexican American), vitamin and supplement use (yes or no), alcohol intake, smoking (serum cotinine), BMI, and serum creatinine.

⁴ Values are adjusted $\bar{x} \pm SE$. Mean comparisons were performed with the Tukey-Cramer test within the food and food group variable if that variable was found significant in the ANCOVA for the Wald *F* test (*P* < 0.05). Means with different superscript letters are significantly different from each other within the food and food group variable, *P* < 0.05 (Tukey-Cramer multiple-comparison test).

⁵ Regression coefficients ± SE (regression analysis).

⁶ Effect of entire food and food group variable in the linear regression model (Wald *F* test).

⁷ Because of the small sample size, 2 groups were collapsed into 1 group.

TABLE 3

Serum total homocysteine (tHcy) concentrations by dietary intakes of folic acid, vitamins B-12 and B-6, and riboflavin in the third National Health and Nutrition Examination Survey¹

Vitamin	Sex- and age-adjusted analysis			Multivariate-adjusted analysis ²		
	Serum tHcy ³	$\beta \pm SE^4$	<i>P</i> for β^5	Serum tHcy ³	$\beta \pm SE^4$	<i>P</i> for β^5
	$\mu\text{mol/L}$			$\mu\text{mol/L}$		
Folic acid (μg)						
<149.3 (<i>n</i> = 1651)	10.5 \pm 0.2 ^a	-0.0019 \pm 0.001	0.0051	10.3 \pm 0.2 ^a	-0.0017 \pm 0.001	0.0044
149.2 to <232.4 (<i>n</i> = 1447)	10.4 \pm 0.3 ^a			10.4 \pm 0.3 ^a		
232.4 to <364.5 (<i>n</i> = 1465)	9.7 \pm 0.2 ^b			9.6 \pm 0.2 ^b		
\geq 364.5 (<i>n</i> = 1433)	9.0 \pm 0.2 ^c			9.2 \pm 0.2 ^b		
Vitamin B-12 (μg)						
<2.05 (<i>n</i> = 1595)	10.2 \pm 0.2	-0.0028 \pm 0.007	0.6988	10.1 \pm 0.2	-0.0035 \pm 0.006	0.5835
2.05 to <3.64 (<i>n</i> = 1534)	10.0 \pm 0.2			10.0 \pm 0.2		
3.64 to <6.19 (<i>n</i> = 1480)	9.6 \pm 0.2			9.7 \pm 0.2		
\geq 6.19 (<i>n</i> = 1387)	9.7 \pm 0.2			9.6 \pm 0.2		
Vitamin B-6 (<i>mg</i>)						
<1.13 (<i>n</i> = 1564)	9.8 \pm 0.3	-0.0077 \pm 0.139	0.9557	9.8 \pm 0.3	0.0505 \pm 0.131	0.7003
1.13 to <1.73 (<i>n</i> = 1588)	10.0 \pm 0.2			10.0 \pm 0.2		
1.73 to <2.5 (<i>n</i> = 1481)	9.8 \pm 0.2			9.8 \pm 0.2		
\geq 2.51 (<i>n</i> = 1363)	9.8 \pm 0.2			9.9 \pm 0.2		
Riboflavin (<i>mg</i>)						
<1.24 (<i>n</i> = 1760)	10.4 \pm 0.3	-0.3122 \pm 0.128	0.0150	10.4 \pm 0.3	-0.2851 \pm 0.129	0.0272
1.24 to <1.81 (<i>n</i> = 1598)	9.8 \pm 0.2			9.8 \pm 0.2		
1.81 to <2.61 (<i>n</i> = 1371)	9.7 \pm 0.2			9.6 \pm 0.2		
\geq 2.61 (<i>n</i> = 1267)	9.6 \pm 0.2			9.6 \pm 0.2		

¹ *n* = 5996. Intakes of dietary vitamins were categorized into quartiles.

² Adjusted for age, sex (male and female), race-ethnicity (non-Hispanic white, non-Hispanic black, and Mexican American), vitamin and supplement use (yes or no), alcohol intake, smoking (serum cotinine), BMI, and serum creatinine.

³ Adjusted $\bar{x} \pm SE$. Mean comparisons were performed with the Tukey-Cramer test within the dietary vitamin intake variable if that variable was found significant in the analysis of covariance for the Wald *F* test (*P* < 0.05). Means with different superscript letters are significantly different from each other within the dietary intake variable, *P* < 0.05 (Tukey-Cramer multiple-comparison test).

⁴ Regression coefficient $\pm SE$ (regression analysis).

⁵ Effect of entire dietary vitamin intake variable in the linear regression model (Wald *F* test).

Another possible explanation is that the poor availability of folate from citrus fruit and juices. Before dietary polyglutamate folate is absorbed, it must be hydrolyzed into monoglutamate folate by intestinal conjugase. It has been shown that organic acids such as citric acid from citrus fruit or juices inhibit intestinal conjugase by lowering the luminal pH (39).


Because vegetables differ in B vitamin content, it is not surprising that the inverse association between tHcy and vegetables was not uniform across the range of vegetables. We found an inverse association only between the frequency of consumption of pepper and cruciferous vegetables and serum tHcy. To our knowledge only one population-based study looked at the association between vegetables and tHcy (21). Using data from the Framingham Study, investigators reported that the consumption of leafy vegetables, including broccoli (average 6.6 compared with 0.3 times/wk), was associated with higher plasma folate and lower tHcy concentrations (21). Dietary guidelines recommend that Americans increase their vegetable consumption from a variety of sources (40). One of the many benefits of vegetable consumption is the increase in intakes of folate and other B vitamins. Although different vegetables have different B vitamin contents, pepper and cruciferous vegetables are generally good sources of folate and other B vitamins. An inverse association between the frequency of consumption of these vegetables and serum tHcy is more likely due to the Hcy-lowering B vitamins

present in these vegetables. However, it is also possible that other components in vegetables may affect tHcy.

Because methionine was positively related to circulating tHcy (41) and was abundant in animal foods (42), we anticipated a positive association between meats, seafood, and eggs and serum tHcy. The lack of association between these foods and serum tHcy is consistent with the observations made by Haulrik et al (42). Besides being abundant in methionine, meats, seafood, and eggs are good sources of riboflavin and vitamins B-6 and B-12. Although speculative, the lack of association between protein-rich foods and serum tHcy may be due to opposite effects of methionine, riboflavin, and vitamins B-6 and B-12 on Hcy metabolism. The lack of relation between the consumption of coffee and serum tHcy agrees with the finding of the Atherosclerosis Risk in Communities study (43) and contradicts with the Hordaland Homocysteine Study (44). In a controlled study, coffee was found to raise circulating tHcy concentrations when subjects drank 1 L (\approx 10–15 cups) paper-filtered coffee containing \approx 1100 mg caffeine (45). In our study, the reported average frequency of coffee intake was \approx 1 time/d (geometric \bar{x} = 31.8 times/mo). The lack of association between tHcy and coffee can be explained by lower intakes of coffee in our study.

Although PLP is required for the transsulfuration of Hcy to cysteine, we found no association between dietary vitamin B-6 and tHcy. Data on vitamin B-6 and tHcy have been equivocal (16,

20, 46–48). Studies have shown that vitamin B-6 had no association with tHcy (20), an inverse association with tHcy in a cross-sectional setting (16), and a tHcy-lowering effect in riboflavin- and folate-replete subjects (46) after the ingestion of a large dose (47) and after a methionine load (48). Our finding of a null association between dietary vitamin B-12 and tHcy agrees with the finding of the Framingham Offspring study (16) and Dutch cohort studies (20). The lack of association between vitamin B-12 and serum tHcy might be related to folate status (49). The average folate intake in this study was 228.7 μg , which is lower than the current recommendation of 400 μg . Although folate intake in NHANES III was underestimated (50), dietary folate intakes in the United States were substantially lower in the prefolate fortification period than after folate fortification (50, 51). Quinlivan and Gregory (51) reported that sequential supplements of 100, 200, and 400 μg folic acid/d diminished its association with tHcy but strengthened the association between tHcy and vitamin B-12. Thus, one might expect either a weak association or a lack of association between tHcy and vitamin B-12 when folate intakes are low.

It is important to note that the food-frequency consumption data we used in this study was qualitative in nature and that the portion sizes were not determined; hence, the quantification of nutrients was not possible. Alternatively, the nutrient values for B vitamins were derived from 24-h food recalls. The strength of this study was that tHcy concentrations were adjusted for various confounding variables. Hence, the observed variation in tHcy may be related to differences in dietary intakes. It is not clearly understood whether elevated circulating tHcy is the cause for the development of vascular disease or is a marker for vascular disease. However, evidence from this study lends support to the importance of including dairy products, cold breakfast cereals, and vegetables (especially peppers and cruciferous vegetables) in one's diet to maintain healthy tHcy concentrations. An increased consumption of these foods will likely improve the intakes of several nutrients and result in Americans meeting their dietary recommendations. 

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REFERENCES

- Nygard O, Vollset SE, Refsum HM. Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. *JAMA* 1995;274:1536–3.
- Riggs SM, Spiro A III, Tucker K, Rush D. Relations of vitamin B-12, vitamin B-6, folate and homocysteine to cognitive performance in the Normative Aging Study. *Am J Clin Nutr* 1996;63:306–14.
- Boushey CJ, Beresford SAA, Wilson PW, Rush D, Rosenberg JH. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. *JAMA* 1995;274:1049–57.
- Stamler JS, Osborne JA, Jaraki O, et al. Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. *J Clin Invest* 1993;91:308–18.
- Majors A, Ehrhart LA, Pezacka EH. Homocysteine as a risk factor for vascular disease. Enhanced collagen production and accumulation by smooth muscle cells. *Arterioscler Thromb Vasc Biol* 1997;17:2074–81.
- Perry IF, Refsum H, Morris RW, Ebrahim SB, Ueland PM, Shaper AG. Prospective study of serum total homocysteine concentration and risk of stroke in middle aged men. *Lancet* 1995;346:1395–8.
- Gerhard GT, Malinow MR, DeLoughery TG, et al. Higher total homocysteine concentrations and lower folate concentrations in premenopausal black women than in premenopausal white women. *Am J Clin Nutr* 1999;70:252–60.
- McCormick DB. Two interconnected B vitamins: riboflavin and pyridoxine. *Physiol Rev* 1989;69:1170–98.
- Schirch L. Serine hydroxymethyltransferase. *Adv Enzymol Related Areas Mol Biol* 1982;53:83–112.
- Jacques PF, Kalmbach R, Bagley PJ, et al. The relationship between riboflavin and plasma total homocysteine in the Framingham offspring cohort is influenced by folate status and the C677T transition in the methylene tetrahydrofolate reductase gene. *J Nutr* 2002;132:283–8.
- Rubba P, Faccenda F, Paucillo P, et al. Early signs of vascular diseases in homocystinuria: a non-invasive study by ultrasound methods in eight families with cystathionine-beta synthase deficiency. *Metabolism* 1990;39:1191–5.
- Saw SM, Yuan JM, Ong CN, et al. Genetic, dietary, and other lifestyle determinants of plasma homocysteine concentrations in middle-aged and older Chinese men and women in Singapore. *Am J Clin Nutr* 2001;73:232–9.
- Selhub J, Jacques PF, Wilson PWF, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *JAMA* 1993;270:2693–8.
- Selhub J, Jacques PF, Rosenberg IH, et al. Serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey (1988–1994): population reference ranges and contribution of vitamin status to high homocysteine concentrations. *Ann Intern Med* 1999;131:331–9.
- Pancharuniti N, Lewis CA, Sauberlich HE, et al. Plasma homocysteine, folate, and vitamin B-12 concentrations and risk for early-onset coronary artery disease. *Am J Clin Nutr* 1994;59:940–8.
- Jacques PF, Bostom AG, Wilson PWF, Rich S, Rosenberg IH, Selhub J. Determinants of plasma total homocysteine concentrations in the Framingham offspring cohort. *Am J Clin Nutr* 2001;73:613–21.
- Ganji V, Kafai RM. Demographic, health, lifestyle, and blood vitamin determinants of serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey, 1988–1994. *Am J Clin Nutr* 2003;77:826–33.
- Chait A, Malinow MR, Nevin DN, et al. Increased dietary micronutrients decrease serum homocysteine concentrations in patients at high risk of cardiovascular disease. *Am J Clin Nutr* 1999;70:881–7.
- Rasmussen LB, Ovesen L, Bulow I, Knudsen N, Laurberg P, Perrild H. Folate intake, lifestyle factors, and homocysteine concentrations in younger and older women. *Am J Clin Nutr* 2000;72:1156–63.
- de Bree A, Verschuren WMM, Blom HJ, Kromhout D. Association between B vitamin intake and plasma homocysteine concentration in the general Dutch population aged 20–65 y. *Am J Clin Nutr* 2001;73:1027–33.
- Tucker KL, Selhub J, Wilson PW, Rosenberg IH. Dietary pattern relates to plasma folate and homocysteine concentrations in the Framingham Heart Study. *J Nutr* 1996;126:3025–31.
- Brouwer IA, Dusseldrop M, West CE, et al. Dietary folate from vegetables and citrus fruit decreases plasma homocysteine concentrations in humans in a dietary controlled trial. *J Nutr* 1999;129:1135–9.
- Malinow MR, Duell B, Irvin-Jones A, Upson BM, Graf EE. Increased plasma homocysteine after withdrawal of ready-to-eat breakfast cereal from the diet: prevention by breakfast cereal providing 200 μg folic acid. *J Am Coll Nutr* 2000;19:452–7.
- Silaste ML, Rantala M, Alfthan G, Aro A, Kesaniemi YA. Plasma homocysteine is decreased by dietary intervention. *Br J Nutr* 2003;89:295–301.
- US Department of Health and Human Services, National Center for Health Statistics. Third National Health and Nutrition Examination Survey, 1988–1994, NHANES III examination data file. Hyattsville, MD: Centers for Disease Control and Prevention, 1996 (CD-ROM). (Public use data file documentation no. 76200.)
- US Department of Health and Human Services, National Center for Health Statistics. Third National Health and Nutrition Examination Survey, 1988–1994, NHANES III household adult data file. Hyattsville, MD: Centers for Disease Control and Prevention, 1996 (CD-ROM). (Public use data file documentation no. 77560.)
- US Department of Health and Human Services, National Center for Health Statistics. Third National Health and Nutrition Examination Survey, 1988–1994, NHANES III second laboratory data file. Hyattsville,

- MD: Centers for Disease Control and Prevention, 1996 (CD-ROM). (Public use data file documentation no. 76300.)
28. US Department of Health and Human Services, National Center for Health Statistics. Third National Health and Nutrition Examination Survey, 1988-1994. NHANES III reference manuals and reports. Hyattsville, MD: Centers for Disease Control and Prevention, 1996 (CD-ROM).
 29. Gunter EW, Lewis BG, Koncikowski SM. Laboratory procedures used for the third National Health and Nutrition Examination Survey, 1988-1994. Hyattsville, MD: US Department of Health and Human Services, Centers for Disease Control and Prevention, 1996.
 30. Jacques PF, Rosenberg IH, Rogers G, et al. Serum total homocysteine concentrations in adolescent and adult Americans: results from the third National Health and Nutrition Examination Survey. *Am J Clin Nutr* 1999;69:483-9.
 31. Araki A, Sako Y. Determination of free and total homocysteine in human plasma by high performance liquid chromatography with fluorescence detection. *J Chromatogr* 1987;422:43-52.
 32. US Department of Agriculture, Agricultural Research Service. Survey nutrient data bases for NHANES III, phase I and phase 2. Riverdale, MD: USDA, 1993 and 1995.
 33. Kleinbaum DG, Kupper LL, Muller KE, Nizam A. Regression diagnostics. In: Kleinbaum DG, Kupper LL, Muller KE, Nizam A, eds. *Applied regression analysis and multivariable methods*. Pacific Grove, CA: Duxbury Press, 1998:212-80.
 34. Riddell LJ, Chisholm A, Williams S, Mann JI. Dietary strategies for lowering homocysteine concentrations. *Am J Clin Nutr* 2000;71:1448-54.
 35. Gregory JF. The bioavailability of folate. In: Bailey LB, ed. *Folate in health and disease*. New York: Marcel Dekker, 1995:195-235.
 36. US Department of Agriculture. Nutrient data base for standard reference, release 16. Washington, DC: US Government Printing Office, 2003.
 37. Powers HJ. Riboflavin (vitamin B-2) and health. *Am J Clin Nutr* 2003;77:1352-60.
 38. Moat SJ, Ashfield-Watt PAL, Powers HJ, Newcombe RG, McDowell IFW. Effect of riboflavin status on the homocysteine-lowering effect of folate in relating to the MTHFR (C677T) genotype. *Clin Chem* 2003;49:295-302.
 39. Tamura T, Shin YS, Buehring KU, Stokstand EL. The availability of folates in man: effect of orange juice supplement on intestinal conjugase. *Br J Hematol* 1976;32:123-33.
 40. Johnson RK, Kennedy E. The 2000 dietary guidelines for Americans: what are the changes and why they were made? *J Am Dietetic Assoc* 2000;100:769-74.
 41. Stolzenberg-Solomon RZ, Miller ER, Maguire MG, Selhub J, Appel LJ. Association of dietary protein intake and coffee consumption with serum homocysteine concentrations in an older population. *Am J Clin Nutr* 1999;69:467-75.
 42. Haulrik N, Toubro S, Dyeberg J, Stender S, Skov AR, Astrup A. Effect of protein and meat intakes on plasma homocysteine concentration: a 6-mo randomized controlled treatment in overweight subjects. *Am J Clin Nutr* 2002;76:1202-6.
 43. Stampfer MJ, Malinow MR, Willett WC, et al. A prospective study of plasma homocysteine and risk of myocardial infarction in US physicians. *JAMA* 1992;268:877-81.
 44. Nygard O, Refsum H, Ueland PM, et al. Coffee consumption and plasma total homocysteine: The Hordaland Homocysteine Study. *Am J Clin Nutr* 1997;65:136-43.
 45. Urgert R, Vliet TV, Zock PL, Katan MB. Heavy coffee consumption and plasma homocysteine: a randomized controlled trial in healthy volunteers. *Am J Clin Nutr* 2000;72:1107-10.
 46. Mckinley MC, McNulty H, McPartlin J, et al. Low-dose vitamin B-6 effectively lowers fasting plasma homocysteine in healthy elderly persons who are folate and riboflavin replete. *Am J Clin Nutr* 2001;73:759-64.
 47. Refsum H, Ueland PM, Nygard O, Vollset SE. Homocysteine and cardiovascular disease. *Annu Rev Med* 1998;49:31-62.
 48. Mansoor MA, Kristensen O, Hervig T, et al. Plasma total homocysteine response to oral dose of folic acid and pyridoxine hydrochloride in healthy individuals. Oral doses of vitamin B-6 reduce concentrations of serum folate. *Scand J Clin Lab Invest* 1999;59:139-46.
 49. Quinlivan EP, McPartlin J, McNulty H, et al. Importance of both folic acid and vitamin B-12 in reduction of risk of vascular disease. *Lancet* 2002;359:227-8.
 50. Institute of Medicine, National Academy of Sciences. *Dietary reference intakes*. Washington, DC: National Academy Press, 1998.
 51. Quinlivan EP, Gregory JF. Effect of food fortification on folic acid intake in the United States. *Am J Clin Nutr* 2003;77:221-5.

