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Association of folate intake and serum homocysteine in elderly persons according to vitamin supplementation and alcohol use[1–3]

Kathleen M Koehler, Richard N Baumgartner, Philip J Garry, Robert H Allen, Sally P Stabler, and Eric B Rimm

ABSTRACT

Background: The serum total homocysteine concentration (tHcy), an indicator of folate status and a possible risk factor for vascular disease, is elevated with impaired renal function and poor vitamin B-12 status, which are common in the elderly.

Objective: Our objective was to determine the association between tHcy, folate intake, alcohol consumption, and other lifestyle factors in elderly persons.

Design: This cross-sectional study used linear regression to model changes in tHcy. Subjects were 278 men and women aged 66–94 y studied in 1993.

Results: Total folate intake was negatively associated with tHcy in models adjusted for age, sex, serum creatinine, and serum albumin. We found an interaction between food folate intake and supplement use. Food folate intake had an inverse dose-response relation with tHcy that was limited to nonusers of supplements. Predicted tHcy was 1.5 μmol/L lower in users of supplements containing folate and vitamin B-12 than in nonusers and was independent of food folate intake. We found a positive dose-response relation of coffee and tea intake with tHcy, a positive association for alcohol intake of ≥60 drinks/mo compared with low intake, and an interaction of alcohol use with folate intake and supplement use. Compared with alcohol users, nonusers had higher predicted tHcy and a lower inverse dose-response relation of food folate intake with tHcy.

Conclusions: The inverse association between folate intake and tHcy was strongest among nonusers of supplements and among alcohol drinkers. Identifying modifiable factors related to tHcy, a possible risk factor for vascular disease, is especially important in elderly persons. *Am J Clin Nutr* 2001;73:628–37.

KEY WORDS Homocysteine, tHcy, folate, folic acid, dietary intake, vitamin supplements, alcohol, coffee, tea, vitamin B-12, methylmalonic acid, elderly

See corresponding editorial on page 501.

INTRODUCTION

The total concentration of the metabolite homocysteine (tHcy) in serum is a sensitive indicator of folate and vitamin B-12 status and may be an independent risk factor for vascular disease (1–4). Folate status itself, measured as dietary intake or serum concentration, is inversely associated with risk of coronary artery disease and stroke (1, 4–7). The metabolic pathway for homocysteine removal by remethylation requires folate and vitamin B-12 and that for removal by catabolism requires vitamin B-6 (8). Individuals with lower intakes or serum concentrations of these 3 B vitamins have higher serum tHcy (9, 10). Conversely, in the absence of vitamin B-12 deficiency, administration of folate alone or together with vitamins B-6 and B-12 significantly lowers tHcy (1, 3, 8, 11, 12). Administration of vitamin B-12 is needed to lower serum tHcy in vitamin B-12–deficient subjects.

Because tHcy is a possible risk factor for vascular disease and a sensitive indicator of folate status, there is interest in understanding the factors related to serum tHcy (9, 10, 13–16). This is true especially for serum tHcy at older ages, when vascular disease risk is increased. Compared with younger adults, elderly persons have a higher prevalence of abnormal renal function and diminished vitamin B-12 absorption. This increases the complexity of the factors related to serum tHcy in elderly persons (17–21) because serum tHcy is also associated with vitamin B-12 deficiency, renal insufficiency, and possibly low vitamin B-6 status.

In an earlier report from the New Mexico Aging Process Study, we showed that serum creatinine, serum folate, vitamin B-12 status, and use of supplements were important in accounting for the variance in serum tHcy (17). In other studies, serum tHcy in middle-aged and older adults also was related to serum vitamin concentrations, dietary vitamin intake, and supplement use (9, 10, 14, 22–24). However, previous studies did not focus on elderly persons (10, 23), had participants who were predominantly supplemented (24), or did not include adjustments for physiologic covariates such as serum creatinine (9, 14, 22).

[1] From the Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC; the Clinical Nutrition Program, University of New Mexico School of Medicine, Albuquerque; the Departments of Medicine and of Biochemistry, Biophysics and Genetics, University of Colorado Health Sciences Center, Denver; the Departments of Epidemiology and of Nutrition, Harvard School of Public Health, Boston; and the Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston.

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[3] Address reprint requests to KM Koehler, Food and Drug Administration, Center for Food Safety and Applied Nutrition, HFS-728, 200 C Street SW, Washington, DC 20204. E-mail: kathleen.koehler@cfsan.fda.gov.

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Alcohol is a known antagonist of folate metabolism (25) and an interaction between alcohol and folate intake was reported in prospective studies of coronary artery disease, colon cancer, and breast cancer (5, 26–28). For each of these diseases, the benefit of folate intake in decreasing risk is stronger in alcohol users than in nonusers. This suggests that alcohol, folate, and some disease processes may be linked by an underlying biological mechanism, which raises the question of whether tHcy may play a role in such a mechanism. Smoking and coffee consumption are additional lifestyle factors that have been associated with tHcy (16, 29, 30) and that should be included in an analysis of tHcy, folate intake, and alcohol use. The purpose of this study was to delineate the relation between tHcy and folate intake from food and supplements in elderly persons and to examine alcohol consumption and other lifestyle factors that may influence the folate-tHcy relation.

SUBJECTS AND METHODS

Subjects

Subjects were participants of the New Mexico Aging Process Study and were studied during their annual data collection visits in 1993. Eligible for inclusion were those originally recruited in 1980 for this prospective study (31) and additional participants recruited over time to replace those who died or left the study for other reasons. Entrance was limited to men and women aged ≥60 y with no known serious medical conditions within the past 5 y. Also excluded were those taking medications for treatment of cardiac or respiratory conditions, those taking antipsychotic medications, and those undergoing chemotherapy. Entrance was not limited to any ethnic group but all volunteers were white; 3% were of Hispanic origin. More than 40% had college degrees. Once accepted into the study, participants were eligible to continue participating regardless of subsequent diagnosis of illness or prescription of medication. Informed consent was obtained from all participants and the study was approved by the Human Research Review Committee of the University of New Mexico School of Medicine.

Dietary and supplement intake

Dietary intake was assessed by using a food-frequency instrument, the Health Habits and History Questionnaire (HHHQ, version 2.2; National Cancer Institute, Bethesda, MD, 1989) (32, 33). The HHHQ was validated against multiple days of dietary intake in previous studies, including studies of elderly persons (34–36). The food list included 3 items for ready-to eat breakfast cereals: highly fortified cereal, bran- or granola-type cereal, and cereal neither highly fortified nor bran or granola type. The 98-item food list was modified slightly to add 4 regional foods (red chile sauce, green chile sauce, enchiladas, and flour tortillas) and low-fat versions of 3 items (cheese, cottage cheese, and salad dressing and mayonnaise).

Nutrient values for the regional foods were added to the food-frequency database as described previously (37, 38) by using FOOD INTAKE ANALYSIS SYSTEM (FIAS) software (version 2.3; US Department of Agriculture and University of Texas, Houston, 1993). Because the food-frequency database did not include vitamin B-12 or methionine, values for these nutrients were added to the database by using the FIAS software for vitamin B-12 values and US Department of Agriculture data (39) for methionine values. Total folate intake was validated against serum folate: for all subjects, $r = 0.64$, $P < 0.0001$; for the supplemented group, $r = 0.33$, $P < 0.01$; and for the unsupplemented group, $r = 0.33$, $P < 0.0001$; these correlations are comparable with those reported for another food-frequency instrument (40).

Questionnaires were administered by an interviewer and covered usual intake during the past year (37). For each item on the food list, participants responded how often they ate the food and whether their portion sizes were small, medium, or large. An open-ended section was used for reporting frequently eaten foods not included on the food list. Completed questionnaires were analyzed with the DIETSYS software package (version 3.7; National Cancer Institute, Bethesda, MD, 1996).

Results of the food-frequency analysis included estimated daily intakes of energy and nutrients, including protein, methionine, folate, vitamin B-6, and vitamin B-12. Additionally, the DIETSYS analysis provided daily intakes of food items and food groups of interest; these data were used together with the DIETSYS nutrient database to calculate the folate intake from ready-to-eat breakfast cereals. Folate from fortified ready-to-eat cereal is predominantly in the form of folic acid. At the time of this study, before the fortification of enriched cereal-grain products with folic acid (41), folate from other foods was primarily naturally occurring folate (3). We calculated folate intake in dietary folate equivalents (DFE) by multiplying folate from ready-to-eat cereals by 1.7 to account for the apparently higher bioavailability of folic acid used in fortification or as a supplement and adding the result to the naturally occurring, noncereal food folate (3). This approach was used previously by Lewis et al (42) to update estimates of folate intake from national surveys.

This analysis also provided intake of coffee and tea combined, as servings per day, from a single item on the questionnaire that included both caffeinated and decaffeinated types. Nutrient intakes were adjusted for energy by using the residual method (43). For use in linear regression models for tHcy, the residuals were added to the expected nutrient value based on the average energy-adjusted nutrient intake of the population. Because folate intake from cereal was found not to be dependent on energy intake ($P = 0.6$), this variable was not energy adjusted.

Of 344 active participants in 1993, 322 completed the food-frequency interview. The completed questionnaires were screened by using the HHHQ editing software. Responses identified as questionable by the software were checked, participants were queried by telephone if necessary, and corrections were made. However, 14 questionnaires were unusable because they had too few foods eaten or too many foods marked "don't know."

As described previously (31), vitamin supplement use was recorded separately in a detailed 3-d record that included brand name, composition, and amount taken for each supplement. Average daily supplemental intake of each vitamin was computed from the 3-d record and was added to vitamin intake from food (from the HHHQ) to provide total daily intake from diet plus supplements. Participants were categorized as taking or not taking supplements according to whether their supplements included all 3 of the B vitamins related to tHcy (folate, vitamin B-6, and vitamin B-12), either as individual supplements or as part of a multivitamin. Because most of those who took supplements of these B vitamins took all 3 vitamins, the analysis could identify associations of tHcy with overall supplement use, but could not attribute the association to one or another specific vitamin. Therefore, those taking only 1 or 2 of the B vitamins were excluded to maintain comparability of vitamin use among supplement users.

Alcohol use was classified as yes or no in answer to the question, "Do you drink alcohol?" Those who reported using alcohol were asked their average number of drinks per month of beer, wine, and liquor over the past year. Total use of alcoholic beverages was calculated as the sum of beer, wine, and liquor use. Physical activity was graded as described previously (44) by using an instrument appropriate for ambulatory, community-dwelling elderly. The instrument assessed usual daily activity, giving a summary score with a possible maximum of 65. For the present study, the range of reported physical activity scores was 0–30.

Serum vitamin and metabolite concentrations

After subjects had fasted overnight, ≈50 mL venous blood was obtained from each subject between 0800 and 0930 for biochemical measurements. Serum samples were stored at –70 °C for 1–10 mo before measurement of vitamin concentrations. Serum and red blood cell folate and serum vitamin B-12 concentrations were measured at the University of New Mexico Clinical Nutrition Laboratory as described previously (17) with a commercially available competitive binding assay and a dual-isotope simultaneous method (SimulTRAC Radioassay Kit, catalog number 262226; Becton Dickinson Advance Diagnostics, Orangeburg, NY). Concentrations of tHcy, methylmalonic acid (MMA), and cystathionine were assayed simultaneously in serum by capillary gas chromatography and mass spectrometry at the University of Colorado Health Sciences Center (8, 45, 46). Serum creatinine, serum albumin, and hematologic indexes were measured and reference ranges were provided by the New Mexico Medical Reference Laboratory as described previously (17).

Statistical analysis

Of the 308 participants for whom dietary intake data were available, metabolite concentrations were not available for 5 and the data for 1 subject were removed from analysis because of a diagnosis of leukemia in this subject near the time of data collection. The analysis was further limited to the 282 participants who were taking or not taking supplements of all 3 of the B vitamins folate, vitamin B-6, and vitamin B-12. Finally, to avoid undue influence on linear models caused by extreme values of independent variables, those with MMA > 1000 nmol/L (n = 1), cystathionine > 3000 μmol/L (n = 2), or vitamin B-12 from food > 20 μg/d (n = 1) were removed, leaving 278 participants for the final analysis. As reported previously (17), the participant with MMA > 1000 nmol/L was a supplemented female with high serum tHcy (21.0 μmol/L), low serum vitamin B-12, and apparent vitamin B-12 deficiency due to an absorption problem.

SAS statistical software (release 6.12; SAS Institute, Inc, Cary, NC, 1996) was used for the analyses. Where appropriate, natural logarithmic transformations were used to approximate normal distributions before carrying out statistical tests and fitting linear models. Chi-square tests of proportions, two-sample t tests, and Pearson correlations (or nonparametric alternatives as noted) were used for descriptive statistics. All tests were two tailed, with a significance criterion of $P < 0.05$.

Multivariable linear regression was used to describe the association of folate intake and supplement use with serum tHcy. Linear regression models used the natural logarithm of serum tHcy to improve normality; energy-adjusted nutrient intake was on the logarithmic scale, as described under dietary intake. In earlier stepwise linear regression models, age, sex, serum creatinine, and serum albumin were important in explaining the variance in serum tHcy (17). We therefore included these variables in our linear models that used folate intake to predict tHcy. We stratified by supplement use (yes or no) to examine possible effect modification of the food folate–tHcy relation and represented the result in a single model by using an indicator variable for supplement use and an interaction term. We calculated predicted serum tHcy for a man aged 76 y with a serum creatinine concentration of 96.8 μmol/L and an albumin concentration of 41.1 g/L by substituting those values for the independent variables in the linear models and varying folate intake and supplement use. We stratified by alcohol use to examine possible effect modification of the supplement–food folate–tHcy relation.

On the basis of our previous results and reports in the literature, we examined the following nutritional and lifestyle factors individually as potential predictors of tHcy in models adjusted for folate intake and supplement use: coffee and tea consumption, cigarette smoking, alcohol use, physical activity score, body mass index, vitamin B-12 status, and intakes of protein, methionine, and vitamins B-6 and B-12. We again calculated predicted tHcy by substituting specific values for these independent variables in the models. We examined the validity of the linear models by using standard diagnostics of residuals (47).

RESULTS

Participant characteristics

Characteristics of the elderly participants are shown in **Table 1**. The subjects' mean age was 76.1 y (range: 66–94 y) and more than one-half were women. About one-half used supplements of all 3 B vitamins; only ≈3% smoked cigarettes. Alcohol users were significantly less likely to be female (55.4% female) than were nonusers (69.8% female). Among alcohol users, mean alcoholic beverage use was modest, 20.7 drinks/mo, corresponding to <1 drink/d. Mean consumption of coffee and tea combined was 2.3 servings/d.

The subjects' mean serum tHcy concentration was 9.28 μmol/L. Age, weight, and height were significantly positively correlated with tHcy. Supplement users had a significantly lower mean serum tHcy than did nonusers (8.29 compared with 10.29 μmol/L). Women had a significantly lower mean (±SD) serum tHcy than did men (8.63 ± 2.45 compared with 10.31 ± 2.78 μmol/L; $P <$ 0.0001). Serum tHcy was significantly positively correlated with serum creatinine, cystathionine, and MMA and significantly negatively correlated with serum and red blood cell folate and serum vitamin B-12.

Alcohol users had a slightly lower mean tHcy than did nonusers (NS), but had significantly higher albumin and hemoglobin concentrations and mean corpuscular volume. For all subjects, there was no correlation between tHcy and alcohol consumption in drinks/mo, but among alcohol users, there was a positive correlation (Spearman's r = 0.16, P = 0.04; not shown).

Dietary intake

The distributions of reported daily intakes of energy and nutrients are shown in **Table 2** (not energy adjusted). Median total folate intake was 460 μg/d and median folate intake from food was 292 μg/d, or 332 μg DFE/d. Median folate intake from ready-to-eat cereal was 59 μg/d, compared with 216 μg/d from foods other than cereals. Eighty-five percent of participants had a food folate intake <400 μg/d and 62% had an intake <320 μg/d

TABLE 1
Characteristics of the elderly participants[1]

Characteristic	All subjects (n = 278)		By supplement use		By alcohol use	
	Value	Correlation with tHcy (r)[2]	No (n = 138)	Yes (n = 140)	No (n = 106)	Yes (n = 168)
Female [n (%)]	170 (61.2)	—	79 (57.3)	91 (65.0)	74 (69.8)	93 (55.4)[3]
Current cigarette smoker [n (%)]	8 (2.9) [275]	—	3 (2.2)	5 (3.6) [137]	1 (1.0) [105]	6 (3.6)
Supplement user [n (%)]	140 (50.4)	—	—	—	52 (49.1)	85 (50.6)
Alcohol user [n (%)]	168 (61.3)	—	—	—	—	—
Age (y)	76.1 ± 6.0	0.27[4]	76.1 ± 6.1	76.0 ± 5.9	77.6 ± 6.5	75.2 ± 5.5[5]
Weight (kg)	68 ± 13	0.20[6]	70 ± 13	67 ± 13[7]	67 ± 13	69 ± 13
Height (cm)	164 ± 10	0.18[6]	164 ± 11	164 ± 9	163 ± 9	165 ± 10
BMI (kg/m^2)	25.2 ± 3.8	0.11	25.8 ± 3.8	24.6 ± 3.7[5]	25.0 ± 3.9	25.2 ± 3.7
Alcohol intake (drinks/mo)	12.7 ± 21.6	0.04	12.8 ± 22.5	12.6 ± 20.7	—	20.7 ± 24.4
Coffee and tea intake (servings/d)	2.3 ± 1.8	0.07	2.3 ± 1.9	2.3 ± 1.7	2.1 ± 1.7	2.4 ± 1.9
Activity score	17.1 ± 5.7	−0.09	17.1 ± 5.7	17.1 ± 5.9	15.8 ± 6.0	18.0 ± 5.3[5]
tHcy (μmol/L)	9.28 ± 2.71	—	10.29 ± 2.99	8.29 ± 1.95[8]	9.46 ± 3.04	9.15 ± 2.50
Cystathionine (nmol/L)	127.0 ± 95.2	0.55[4]	150.2 ± 117.4	104.2 ± 58.3[9]	131.6 ± 93.5	125.0 ± 97.2
MMA (nmol/L)	164.9 ± 79.3	0.39[4]	183.2 ± 90.9	146.9 ± 61.2[10]	167.3 ± 67.6	164.0 ± 86.3
Folate (nmol/L)	33.5 ± 14.3	−0.51[4]	24.9 ± 10.2	42.0 ± 12.7[8]	34.0 ± 15.0	33.3 ± 13.9
RBC folate (nmol/L)	1343 ± 473 [274]	−0.30[4]	1128 ± 396	1562 ± 445[8] [136]	1371 ± 498 [105]	1324 ± 459 [165]
Vitamin B-12 (pmol/L)	354 ± 178	−0.45[4]	279 ± 101	428 ± 204[11]	353 ± 195	354 ± 167
Creatinine (μmol/L)	96.5 ± 19.0	0.50[4]	99.7 ± 21.0	93.5 ± 16.4[5]	96.2 ± 21.5	96.8 ± 17.5
Albumin (g/L)	41.1 ± 2.6	0.08	41.1 ± 2.5	41.1 ± 2.8	40.6 ± 2.6	41.3 ± 2.6[7]
Hemoglobin (g/L)	147 ± 13	0.09	146 ± 14	147 ± 13	144 ± 14	148 ± 13[5]
MCV (fL)	91.2 ± 4.4	−0.06	90.3 ± 4.7	92.1 ± 3.9[5]	90.3 ± 4.9	91.7 ± 4.0[7]

[1] $\bar{x}$ ± SD; n in brackets. Supplement use was defined as taking supplements of all 3 of the B vitamins folate, vitamin B-6, and vitamin B-12, either separately or as part of a multivitamin. Alcohol intake was defined as yes or no in answer to the question, "Do you use alcohol?" tHcy, total homocysteine concentration; MMA, methylmalonic acid; RBC, red blood cell; MCV, mean corpuscular volume.

[2] Pearson correlation coefficient for ln(tHcy) with variable [or with ln(variable) for cystathionine, MMA, and vitamin B-12], except Spearman correlation coefficient for ln(tHcy) with alcoholic beverages and coffee and tea.

[3] Significantly different from no by sex, $P < 0.05$ (chi-square test of proportions).

[4] $P < 0.001$.

[6] $P < 0.01$.

[5,7,8] Significantly different from no (t test of means): [5] $P < 0.01$, [7] $P < 0.05$, [8] $P < 0.0001$.

[9–11] Significantly different from no (t test of geometric means): [9] $P < 0.0001$, [10] $P < 0.001$, [11] $P < 0.01$.

(not shown). When expressed as DFE, 69% of participants had a food folate intake <400 μg DFE/d [the 1998 recommended dietary allowance (RDA)] and 48% had an intake <320 μg DFE/d [the 1998 estimated average requirement (EAR)] (not shown) (3).

The most common supplemental folate dose was 400 μg/d, which was taken by 97 (69.3%) of the supplemented participants; 18 participants (6.5%) took >400 μg/d. The common supplemental vitamin B-12 doses were 6, 9, and 25 μg/d, taken by 58 (41.4%), 18 (12.9%), and 16 (11.4%) of supplemented participants, respectively; 21 participants (7.5%) took >25 μg/d. The common vitamin B-6 supplement doses were 2 and 3 mg/d, taken by 64 (45.7%) and 26 (18.6%) of supplemented participants, respectively; 14 participants (5%) took >35 mg/d. After energy adjustment, there was a strong negative correlation of serum tHcy with total folate intake ($r = -0.42$) and weaker negative correlations with food folate intake ($r = -0.16$) and food vitamin B-6 intake ($r = -0.15$) (Table 2).

Linear regression models

Shown in **Table 3** is the linear regression model for serum tHcy, showing a highly significant negative coefficient for total folate intake. Age, male sex, serum creatinine, and serum albumin had significant positive coefficients and the model explained 42% of the variance in serum tHcy. The predicted serum tHcy concentration across a range of total folate intake, with the other variables held constant, is illustrated in **Figure 1**.

In linear regression models for serum tHcy stratified by supplement use, the negative coefficient for food folate intake was highly significant in the model for the unsupplemented group but was not a significant predictor for the supplemented participants (**Figure 2**). The interaction between food folate intake and supplement use, shown in Figure 2, is represented in a single model by adding an indicator variable for supplement use (0 = no supplement use, 1 = supplement use) and an interaction term for food folate intake × supplement use. This model explained 43% of the variance in serum tHcy. The coefficients for age, male sex, serum creatinine, and serum albumin were all significant at $P < 0.05$, as was the food folate × supplement use interaction term. The changes in predicted serum tHcy corresponding to changes in the independent variables in this interaction model are shown in **Table 4**.

Smoking, coffee and tea intake, and alcohol intake

We assessed cigarette smoking, coffee and tea intake, and alcohol consumption as possible predictors of tHcy by using this interaction model. As shown in Table 4, cigarette smoking was associated with an increased serum tHcy of 1.68 μmol/L. Coffee and tea consumption was significant in the model and predicted an increase in tHcy. When coffee and tea intake was represented in

TABLE 2
Dietary intake of the elderly participants[1]

Daily dietary intake	Percentile					Correlation with tHcy (r)[2]
	10th	25th	50th (median)	75th	90th	
Energy (MJ)	4428	5147	6322	7794	9808	0.13[3]
Folate						
Total intake (μg)	206	288	460	681	818	−0.42[4]
Food intake						
(μg)	180	226	292	357	435	−0.16[5]
(μg DFE)[6]	197	250	332	424	532	
Noncereal (μg)	138	175	216	264	329	−0.06
Cereal (μg)	1	24	59	103	156	−0.10
Vitamin B-6						
Total (mg)	1.19	1.51	3.06	4.45	11.97	−0.34[4]
Food (mg)	1.05	1.31	1.56	1.94	2.4	−0.15[3]
Vitamin B-12						
Total (μg)	2.51	4.05	8.50	13.70	28.60	−0.35[4]
Food (μg)	2.08	2.78	3.93	5.25	7.54	0.01
Protein (g)	37.5	46.8	58.1	72.3	85.0	−0.11
Methionine (mg)	827	1027	1299	1592	1916	−0.09

[1] n = 278. Median and other percentiles shown are not energy adjusted, but data were energy adjusted for calculation of correlation coefficients. tHcy, total homocysteine concentration.
[2] Pearson correlation coefficient for ln(tHcy) with ln(energy-adjusted dietary intake). Intake adjusted for energy intake by residual method except for cereal folate, which was not energy adjusted because it was independent of energy intake (*see* text).
[3] $P < 0.05$.
[4] $P < 0.0001$.
[5] $P < 0.01$.
[6] DFE, dietary folate equivalents. 1 μg DFE = 1 μg naturally occurring food folate (noncereal folate) = 0.6 μg added folic acid from fortification (cereal folate) (3).

4 groups by using indicator variables, the model predicted significantly lower serum tHcy in the 2 lowest consumption groups than in those consuming >3 servings/d; a dose-response relation was confirmed by a significant test for linear trend. Results were similar in models restricted to nonsmokers (data not shown).

When alcohol intake was represented in 4 groups by using indicator variables, the model predicted a tHcy ≈1.3 μmol/L higher for alcohol use at high intakes than for alcohol use at low intakes (Table 4). The test for linear trend was not significant. When the model was adjusted for coffee and tea intake or restricted to nonsmokers, the results were essentially unchanged (data not shown).

To examine further the relation of alcohol intake with serum tHcy, the sample was stratified by alcohol use (yes or no). For those taking supplements, the predictions for alcohol users and nonusers were not appreciably different, with minimal change in predicted serum tHcy across the range of food folate intake. For unsupplemented participants, alcohol users had both a lower predicted serum tHcy at an average food folate intake and a steeper decrease in predicted serum tHcy with increasing food folate intake than did alcohol nonusers (**Figure 3**). For alcohol users, the coefficients for food folate, supplement use, and the food folate intake × supplement use interaction had P values of 0.0003, 0.005, and 0.009, respectively. However, for alcohol nonusers, the respective coefficients were not significant (P = 0.19, 0.27, and 0.16, respectively). Further control for amount of alcohol use among users did not appreciably change these results.

Vitamin B-12 and other covariates

The serum MMA concentration was significantly positively associated and the serum vitamin B-12 concentration was significantly negatively associated with serum tHcy (Table 4). In models adjusted for serum MMA, the association of tHcy with food folate intake and supplement use was essentially unchanged (not shown). In the model adjusted for serum vitamin B-12, coefficients for food folate intake, supplement use, and the food folate intake × supplement use interaction remained significant ($P < 0.01$) but somewhat attenuated (the predicted tHcy decrease was 0.83 μmol/L with supplement use and 0.45 μmol/L with an increase in food folate of 100 μg/d). When the models stratified by alcohol use were adjusted for serum vitamin B-12 or MMA, or when those with elevated MMA were omitted (n = 25), the apparent interactions of alcohol use with food folate intake and supplement use were essentially unchanged from the unadjusted models in Figure 3 (data not shown).

Both food vitamin B-12 intake (with a food vitamin B-12 × supplement use interaction) and protein intake were negatively

TABLE 3
Linear regression model predicting ln(tHcy) using total folate intake[1]

Independent variable	Regression coefficient	SE	P
Intercept	1.3911	0.3328	0.0001
Total folate [ln(mg/d)][2]	−0.1698	0.0234	0.0001
Age (y)	0.0108	0.0022	0.0001
Male sex[3]	0.0837	0.0298	0.005
Serum creatinine (μmol/L)	0.0049	0.0008	0.0001
Serum albumin (g/L)	0.0124	0.0049	0.01

[1] n = 278. Adjusted R^2 = 0.42. tHcy, total homocysteine concentration.
[2] Total folate: ln(total folate intake from food plus supplements, μg/d), adjusted for energy intake by residual method.
[3] Indicator variable: yes = 1, no = 0.

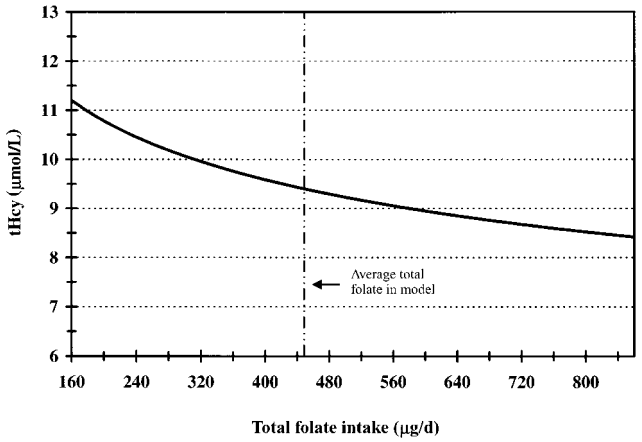

FIGURE 1. Predicted total homocysteine concentration (tHcy) in serum according to total folate intake from food and supplements (n = 278). Predicted tHcy is from the model in Table 3 for a man aged 76 y with a serum creatinine concentration of 96.8 μmol/L and a serum albumin concentration of 41.1 g/L. Folate intake was adjusted for energy by using the residual method. Average energy-adjusted food folate intake for the population was 446 μg/d.

associated with serum tHcy (P = 0.08) in the interaction model (Table 4), but adjustment for these variables did not appreciably change the association of food folate intake or supplement use in the model (not shown). Methionine intake was slightly less significant when it replaced protein in the model. BMI, activity level, and food vitamin B-6 intake were not significant in the model (not shown).

Cereal and noncereal folate

As shown in Table 4, when included together in the model in place of food folate intake, cereal and noncereal folate intake were significantly negatively associated with tHcy and each showed a significant folate intake × supplement use interaction. For supplemented participants, there was little change in predicted tHcy across the range of cereal or noncereal folate intake (data not shown). In contrast, for unsupplemented participants, a 100-μg/d increase in cereal or noncereal folate intake predicted decreases in tHcy of 0.4 and 0.6 μmol/L, respectively, roughly similar to that seen in the original model with use of food folate (Table 4). When the sample was stratified by alcohol use and the cereal and noncereal folate models were refitted, an interaction between noncereal folate and alcohol use was observed, similar to that seen for food folate in Figure 3. In comparison with noncereal folate, the association of cereal folate with serum tHcy was similar in models for all subjects (Table 4) or when stratified by alcohol use (data not shown).

DISCUSSION

In this population of 278 elderly persons, we found that although total folate intake was negatively associated with serum tHcy, the inverse dose-response association for food folate intake was limited to nonusers of supplements. For supplement users, serum tHcy was lower and was independent of food folate intake. We also found a positive dose-response relation of coffee and tea intake with tHcy. There were 2 relations of alcohol intake with tHcy: a positive association for intake ≥60 drinks/mo compared with low intake, and effect modification of folate intake and supplementation for alcohol use or nonuse. Alcohol nonusers had higher predicted serum tHcy and the inverse dose-response relation with food folate intake was smaller and less significant than in alcohol users.

Because it was cross-sectional, this study could identify associations with serum tHcy but could not show causality. The ability of supplemental or fortification folic acid or B vitamins to lower tHcy was shown in several studies (1, 3, 11, 12, 48–50). Our study describes the prediction of tHcy over a range of folate intakes, including the role of naturally occurring food folate, which is not readily observed in randomized trials. Furthermore, this study included the lifestyle factors of alcohol use and coffee and tea intake, as well as vitamin B-12 status, serum creatinine, and serum albumin, factors particularly important in elderly persons. Participants were relatively health-conscious volunteers in a prospective cohort (17, 31) and therefore the results may not apply to the general population. However, the participants had a broad age range and the results were generally consistent with those from other volunteer cohorts (9, 18, 24) and thus can probably be generalized to other active, elderly populations in the United States.

The potential for misclassification is inherent in dietary intake measurement, and databases may underestimate the naturally occurring folate in food (3). However, folate intake was validated against serum folate and food, cereal, and noncereal folate all had significant relations with tHcy. We did not measure genetic variants of the methylene tetrahydrofolate reductase enzyme (4). However, any error resulting from this omission would be random and would be expected to bias results toward the null.

At lower ranges of serum folate, tHcy decreases as serum folate increases, but at higher serum folate, tHcy reaches a low plateau and remains the same as serum folate increases (1, 3, 9, 51). We showed previously a serum folate × supplement interaction in

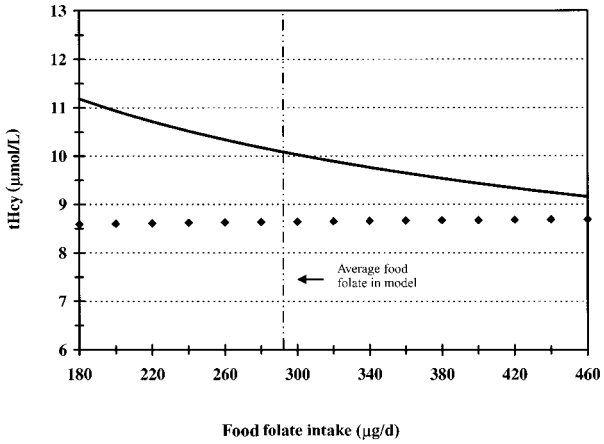

FIGURE 2. Predicted total homocysteine concentration (tHcy) in serum according to folate intake from food in 138 unsupplemented (solid line) and 140 supplemented (diamonds) participants. Predicted tHcy is the result of stratifying the model in Table 3 by supplement use and replacing total folate intake with food folate intake for a man aged 76 y with a serum creatinine concentration of 96.8 μmol/L and a serum albumin concentration of 41.1 g/L. Supplement use was defined as taking self-selected supplements of all 3 of the B vitamins folate, vitamin B-6, and vitamin B-12. Folate intake was adjusted for energy by using the residual method. Average energy-adjusted food folate intake for the population was 291 μg/d. Adjusted R^2: 0.38 for supplemented, 0.29 for unsupplemented.

TABLE 4
Changes in predicted serum tHcy with changes in independent variables in regression models[1]

Independent variable	Change in variable[2]	Change in predicted tHcy[3]	*P*
		μmol/L	
Food folate model[4]			
Age (y)	−5	−0.55	0.0001
Sex	Female	−0.74	0.01
Creatinine (μmol/L)	−10	−0.50	0.0001
Albumin (g/L)	−5	−0.62	0.01
Taking supplments	Yes	−1.52	0.004
Food folate (μg/d)[5]	100	−0.61	0.0005
	−100	0.95	0.0005
Food folate × supplement interaction	—	—	0.01
Food folate model with added variables[6]			
Cigarette smoking	Yes	1.68	0.04
Coffee and tea intake (servings/d)			
Indicator variables	≤1	−1.00	0.005
	>1, ≤2	−0.77	0.03
	>2, ≤3	−0.59	0.15
	>3	Referent	—
Test for linear trend[7]	—	—	0.004
Alcohol intake (drinks/mo)			
Indicator variables	0	0.23	0.44
	>0, <30	Referent	—
	≥30, <60	−0.13	0.74
	≥60	1.26	0.03
Test for linear trend[8]	—	—	NS
Serum vitamin B-12 (pmol/L)[5,9]	−85	0.60	0.0001
Serum MMA (nmol/L)[5,9]	60	0.48	0.0001
Serum MMA >271 nmol/L	Yes	1.49	0.002
Protein intake (g/d)[5,9]	10	−0.22	0.08
Vitamin B-12 intake (μg/d)[5,9]	3	−0.45	0.08
Vitamin B-12 × supplement interaction	—	—	0.18
Cereal and noncereal folate model[10]			
Noncereal folate (μg/d)[5]	100	−0.60	0.01
Noncereal folate × supplement interaction	—	—	0.02
Cereal folate (μg/d)[5]	100	−0.38	0.002
Ceral folate × supplement interaction	—	—	0.003

[1] n = 278, except n = 275 with cigarette smoking added to model and n = 274 with alcohol intake added to model. tHcy, total homocysteine concentration.

[2] Change in value of independent variables in linear regression model to predict corresponding change in tHcy.

[3] Change in predicted tHcy corresponding to change in value of independent variable according to model; predicted values are antilogarithms from models predicting ln(tHcy).

[4] The independent variables were included together in the model. Predicted tHcy was 10.13 μmol/L for an unsupplemented man aged 76 y, with serum creatinine of 96.8 μmol/L, albumin of 41.1 g/L, and food folate intake of 291 μg/d. Adjusted R^2 = 0.43.

[5] Model used ln of continuous variable.

[6] Variables added separately to food folate model. With additional variables in model, adjusted R^2 = 0.43 or 0.44, except with serum MMA, for which adjusted R^2 = 0.46, and with serum vitamin B-12, for which adjusted R^2 = 0.51.

(Continued)

TABLE 4 (*Continued*)

[7] In test for linear trend, intake of group was represented by that group's mean: 0.5, 1.8, 2.8, and 5 servings/d, respectively (n = 86, 81, 51, and 60, respectively).

[8] In test for linear trend, intake of group was represented by ordinal numbers 1–4 (n = 106, 115, 35, and 18, respectively).

[9] Changes in continuous variables compared, respectively, with serum vitamin B-12, 315 pmol/L; serum MMA, 150 nmol/L; protein intake, 60.2 g/d; or vitamin B-12 intake, 4 μg/d.

[10] Cereal and noncereal folate and interaction terms replaced food folate and interaction terms in food folate model. Cereal folate not adjusted for energy intake because it was independent of energy intake (*see* text). For noncereal folate intake of 220 μg/d and cereal folate intake of 29.5 μg/d, predicted tHcy was 10.10 μmol/L. Adjusted R^2 = 0.43.

predicting tHcy (17), similar to the present food folate intake × supplement interaction (Figure 2). In the elderly Framingham cohort, there was a low tHcy plateau at a higher total folate intake (9) and in an elderly Baltimore cohort, there was a significant food folate intake × supplement interaction (24). However, in that study, few subjects were unsupplemented and the negative food folate coefficient was not significant (24).

Intake of coffee and tea and of alcohol

Two previous studies, conducted in Hordaland, Norway (30), and Baltimore (24), reported positive associations of coffee intake with serum tHcy, but none were found in the Atherosclerosis Risk in Communities (ARIC) study (52). As in the Hordaland study, the association in our study was retained in models restricted to nonsmokers. The mechanism of this association is unknown and the reason for the inconsistency among reports is uncertain. In our study, adjustment for serum creatinine may have helped us to detect an association. The simple correlation between tHcy and coffee and tea intake was minimal (Table 1; r = 0.07, P = 0.27), but was strengthened with successive adjustment for age and sex (r = 0.10, P = 0.10), creatinine and albumin (r = 0.11, P = 0.07), and supplement use (r = 0.13, P = 0.02).

Although excessive alcohol intake is associated with impaired folate status, reports of association of alcohol intake with serum tHcy are inconsistent (10, 53, 54). Elevated serum tHcy was reported among chronic alcoholics (55, 56), consistent with the positive association with higher alcohol use in our study. Chronic alcoholics have lower serum concentrations of vitamins B-12 and B-6 and lower red blood cell folate than do control subjects, but serum folate is not significantly different (56). In our study, those with higher alcohol intakes had significantly lower serum folate but not red blood cell folate or serum vitamin B-12. We are not aware of previous reports of an interaction between alcohol use and food folate intake or supplement use in predicting serum tHcy (Figure 3). However, an interaction between alcohol and folate intake was seen in cohort studies of coronary artery disease, colon cancer, and breast cancer (5, 26–28). In all cases, folate was more beneficial in alcohol users than in nonusers; a biological mechanism to explain this interaction has not been adequately addressed. In our study, alcohol nonusers were more likely to be female and older and were apparently more frail, with a lower activity score, mean corpuscular volume, serum albumin, and hemoglobin (Table 1). However, the models were adjusted for age, sex, serum creatinine, and serum albumin, suggesting that the interaction was truly associated with alcohol use.

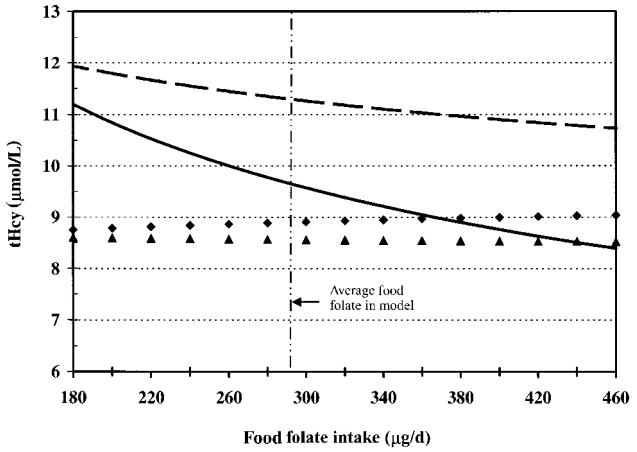

FIGURE 3. Predicted total homocysteine concentration (tHcy) in serum according to folate intake from food for 168 unsupplemented (solid line) and supplemented (triangles) alcohol users and for 106 unsupplemented (dashed line) and supplemented (diamonds) alcohol nonusers. Predicted tHcy is for a man aged 76 y with a serum creatinine concentration of 96.8 μmol/L and a serum albumin concentration of 41.1 g/L. Models were stratified by alcohol intake. Models included an indicator variable for supplement use and a food folate intake × supplement use interaction term. Supplement use was defined as taking self-selected supplements of all 3 of the B vitamins folate, vitamin B-6, and vitamin B-12. Folate intake was adjusted for energy intake by using the residual method. Average energy-adjusted food folate intake for the population was 291 μg/d. Adjusted R^2: 0.52 for alcohol nonusers, 0.38 for alcohol users.

Vitamin B-12

In previous studies, serum vitamin B-12 was a negative predictor and serum MMA was a positive predictor of serum tHcy after adjustment for serum folate (9, 15, 17, 57). Although we excluded one participant with apparent severe vitamin B-12 deficiency (*see* Methods), serum vitamin B-12 and MMA were significant predictors of tHcy (Table 4). Adjustment for serum vitamin B-12 accounted for about one-half of the decrease in predicted tHcy with supplement use, indicating that both the vitamin B-12 and the folate from supplements were contributing to the decrease in serum tHcy. In a middle-aged cohort whose folate status improved during the implementation of folic acid fortification, the remaining difference in serum tHcy associated with supplements was explained by adjustment for serum vitamin B-12 and vitamin B-6 (48). In a clinical trial, the decrease in serum tHcy with folate supplementation was successively greater with the addition of 6 and 400 μg vitamin B-12/d (11). Generous amounts of folate and vitamin B-12 may act synergistically to decrease serum tHcy by promoting enzymatic removal (11). As reported in the Baltimore study (24), we found an inverse association of dietary protein and tHcy (Table 4). High-protein diets probably increase the removal of tHcy through the catabolic, transsulfuration pathway, independent of remethylation with folate and vitamin-B-12 (24, 58).

Cereal and noncereal folate

The models did not correct for either the possibly greater bioavailability of fortification folic acid or the possible underestimation of naturally occurring food folate (3) and we found that cereal and noncereal folate intakes predicted roughly comparable changes in tHcy (Table 4). Because the range of cereal folate intake was small (Table 2) and cereal also provided crystalline vitamins B-12 and B-6 (59), our ability to detect differences between these sources may have been limited. However, the analysis did show that although cereal folate accounted for ≈26% of the food folate intake in our sample (37), naturally occurring food folate intake was an important predictor of serum tHcy in unsupplemented elderly persons. This is in contrast with the ARIC study, in which breakfast cereal accounted for most of the ability of food folate intake to predict tHcy (23). Our regression model had separate terms for cereal and noncereal folate, whereas the ARIC model used terms for total food folate and cereal servings. The ability of specific folate sources, such as fruit and vegetables, to predict tHcy was shown earlier (14, 23).

The 1998 EAR and RDA aim to maintain biomarkers of adequate folate status, including serum tHcy (3). The percentage of a population below the EAR for a nutrient represents the proportion at risk of inadequate intake (3). In our study, before the fortification of cereal-grain products with folic acid, food folate intake below the EAR and RDA was common (Table 2), consistent with the model predictions that serum tHcy could be lowered by increased folate from food or supplements (Table 4 and Figure 2).

Conclusions

We found an inverse association of serum tHcy with food folate intake in nonusers of supplements, but no relation for supplement users, confirming that tHcy reaches a low plateau at higher folate status. Alcohol use interacted with folate intake and supplement use in models for tHcy, suggesting that a previously described interaction of alcohol use and folate intake with coronary artery disease risk may involve tHcy (5). Identification of potentially modifiable factors related to tHcy in elderly persons is especially important because vascular disease is more prevalent in the elderly and because higher tHcy is associated with vitamin B-12 deficiency and impaired renal function, more common at older ages.

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