Association of dietary protein intake and coffee consumption with serum homocysteine concentrations in an older population[1–3]

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ABSTRACT

Background: Elevated blood concentrations of total homocysteine (tHcy) have been implicated in the pathogenesis of atherosclerotic cardiovascular disease. Previous studies identified suboptimal nutritional status and dietary intake of folate, vitamin B-6, and vitamin B-12 as determinants of elevated tHcy.

Objective: We identified other nutritional factors associated with tHcy in 260 retired schoolteachers in the Baltimore metropolitan area.

Design: We performed observational analyses of baseline and 2–4-mo follow-up data collected in a study designed to test the feasibility of conducting a large-scale clinical trial of vitamin supplements by mail. The study population consisted of 151 women and 109 men with a median age of 64 y. At baseline, each participant completed a food-frequency questionnaire. At follow-up, fasting serum tHcy was measured.

Results: In multivariable linear regression and generalized linear models, there was an independent, inverse dose-response relation between dietary protein and ln tHcy ($P = 0.002$) and a positive, significant dose-response relation between coffee consumption and ln tHcy (P for trend = 0.01). Other significant predictors of ln tHcy were creatinine (positive; $P = 0.0001$) and prestudy use of supplemental B vitamins (inverse; $P = 0.03$). In stratified analyses restricted to persons receiving standard multivitamin therapy, the association of ln tHcy with dietary protein and coffee persisted.

Conclusions: These results support the hypothesis that increased protein intake and decreased coffee consumption may reduce tHcy and potentially prevent atherosclerotic cardiovascular disease and other disease outcomes. *Am J Clin Nutr* 1999; 69:467–75.

KEY WORDS Homocysteine, tHcy, diet, protein, coffee, folic acid, vitamin B-12, creatinine, vitamin supplements, observational study, elderly

INTRODUCTION

Numerous observational studies have shown an association between elevated total homocysteine (tHcy) concentrations in blood and an increased risk of cardiovascular disease (1, 2). This relation appears to be independent of other coronary artery disease risk factors and is graded throughout the entire range of tHcy concentrations (3). Studies that measured plasma homocysteine after methionine loading showed similar results (4, 5). Higher concentrations of tHcy have also been associated with cognitive impairment in older persons (6) and with birth defects in pregnant women (7–9).

Several factors have been associated with elevated blood homocysteine concentrations in humans (10). Blood concentrations and dietary intake of folate and vitamin B-6 and blood concentrations of vitamin B-12 are inversely related to elevated plasma homocysteine concentrations (11). Dietary vitamin B-12, in contrast, has not been consistently related to lower tHcy concentrations (12, 13). These associations are consistent with homocysteine metabolism [**Figure 1** (14, 15)]. Homocysteine is either remethylated to methionine predominantly by methionine synthase (5-methyltetrahydrofolate–homocysteine *S*-methyltransferase) in a reaction that requires methyltetrahydrofolate (as a methyl donor) and vitamin B-12 (as an enzyme cofactor) or is transsulfurated to cysteine in reactions that require pyridoxal-*P*, the coenzyme form of vitamin B-6 (15). Persons homozygous for thermolabile methylenetetrahydrofolate reductase deficiency have mild hyperhomocysteinemia and a greater risk of coronary artery disease, which can be modified with folate in the diet or from supplements (11, 16, 17). Persons heterozygous for cystathionine β-synthase deficiency also have mild hyperhomocysteinemia that can be modified with vitamin B-6 and betaine supplementation; however, heterozygous cystathionine β-synthase deficiency has not been consistently linked with vascular disease

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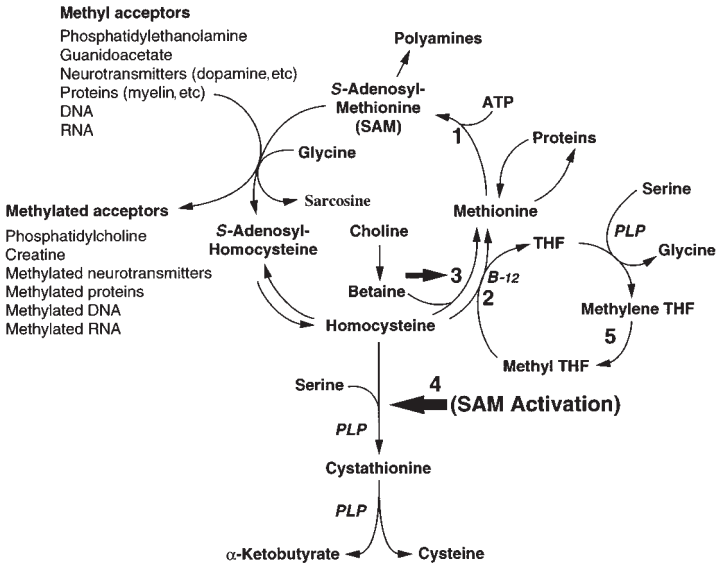

FIGURE 1. Homocysteine metabolism in humans. The enzyme reactions that are activated by *S*-adenosyl-methionine (SAM) are indicated by the large arrows (14). Enzymes involved in each reaction are as follows: *1*) methionine adenosyltransferase; *2*) methionine synthase (5-methyltetrahydrofolate–homocysteine *S*-methyltransferase); *3*) betaine–homocysteine *S*-methyltransferase; *4*) cystathionine β-synthase; and *5*) 5,10-methylenetetrahydrofolate reductase. B-12, vitamin B-12; PLP, pyridoxal-*P*; THF, tetrahydrofolate. Adapted with permission from *Present Knowledge in Nutrition*, 7th ed (15). ©1996 by the International Life Sciences Institute, Washington, DC. All rights reserved.

(11, 18–20). The frequency of thermolabile methylenetetrahydrofolate reductase deficiency is 5–12% in the general North American population (17, 21–23). Older age, male sex, impaired renal function, cigarette smoking, high alcohol intake, and, in one recent report, coffee consumption are also associated with greater tHcy concentrations; multivitamin use and dietary riboflavin are associated with lower tHcy concentrations (11, 13, 24–28). The purpose of this paper was to identify other nutritional factors associated with tHcy in a cohort of retired schoolteachers in the Baltimore vicinity.

SUBJECTS AND METHODS

The data presented in this paper resulted from observational analyses of baseline and follow-up data collected from participants in the Vitamin, Teachers, and Longevity (VITAL) pilot study, which was designed to assess the feasibility of conducting a large-scale clinical trial of antioxidant vitamin supplements by mail. The study was approved by an institutional review board at the Johns Hopkins Medical Institutions. All participants provided written, informed consent.

Study participants

The participants were members of the Baltimore City, Baltimore County, and Anne Arundel County affiliates of the Maryland Retired Teachers Association. In November 1993, 4774 retired teachers received an invitation by mail to participate in the pilot study. Inclusion criteria included a willingness to discontinue vitamin A, β-carotene, vitamin C, and vitamin E use; to be randomly allocated an antioxidant vitamin preparation or a placebo; and, if taking a multivitamin supplement, to replace it with a standard multivitamin provided by the study. Persons receiving coumadin or participating in other trials were excluded. In January 1994, interested and eligible persons who received the first mailings were sent a medical history questionnaire and a food-frequency questionnaire. Two-hundred ninety-seven men and women who remained interested and who fulfilled these requirements by the target date (mid-March 1994) were enrolled in the study.

Design

Participants were randomly assigned to receive either an antioxidant vitamin preparation or a matching placebo. The antioxidant vitamin preparation contained 400 IU α-tocopherol, 500 mg vitamin C, 6 mg β-carotene, 15 mg Zn, 15 μg Se, 2 mg Cu, and 3 mg Mn. Persons who requested multivitamin therapy were provided with a standard multivitamin (Centrum; Lederle, Pearl River, NY) that contained 400 μg folic acid, 6 μg vitamin B-12, and 2 mg vitamin B-6 along with other vitamins and minerals. These participants were told to take no more than one multivitamin daily. Eighty-five percent of the intervention and placebo group participants requested the standard multivitamin.

In April 1994, participants were mailed the randomly assigned pills (antioxidant preparation or placebo) and standard multivitamins (if requested). After 2–4 mo, participants attended a follow-up clinic visit at which blood pressure, height, and weight were measured; a fasting blood sample was obtained; remaining pills were counted; and additional questionnaires were completed. Standard methods and quality-control procedures were used for all measurements. Height, weight, and blood pressure measurements were performed by trained and certified personnel with a stadiometer, calibrated balance-beam scale, and standard mercury manometer, respectively. The trial was double blinded.

Diet history

Before the intervention, participants completed the 1992 version of the Health Habits and History Questionnaire (HHHQ), developed at the National Cancer Institute by Block et al (29). This semiquantitative food-frequency questionnaire assessed usual food intake, vitamin and mineral intake, and portion sizes

for the past 12 mo. For each food, a commonly used medium portion size was specified and participants were asked how often they consumed the food and how much they consumed. There were 9 possible frequency responses, which ranged from never to ≥2 servings/d, and 3 serving sizes (small, medium, and large). The HHHQ included questions on the frequency and brand of both multivitamin and individual vitamin supplements. Both the food list and nutrient values for the questionnaire were based on data from the second National Health and Nutrition Examination Survey (1976–1980). The questionnaire was not modified for regional foods. It was self-administered after participants had expressed interest in the study.

The questionnaire was subsequently scanned and the daily intake of various nutrients was determined by using HHHQ-DIETSYS software (version 4.0, 1996; National Cancer Institute, Bethesda, MD). DIETSYS software was also used to determine the top food sources of protein. Nutrients derived from the HHHQ and DIETSYS software and used in the analyses included dietary energy, protein, folate, vitamin B-6, and riboflavin; supplemental folic acid, vitamin B-6, and vitamin B-12; and percentage of energy from alcohol, protein, carbohydrate, and fat. The analysis software did not estimate dietary vitamin B-12 or coffee consumption or discriminate between protein from animal or vegetable sources.

Self-reported coffee consumption was determined from the open-ended question, "How may cups of coffee do you drink on a typical day?," which was asked on a study questionnaire given to participants during their follow-up visit. Vitamin supplement use was described 2 ways (each coded as yes or no): *1*) provision of the standard multivitamin during the intervention and *2*) prestudy use of supplemental B vitamins (folic acid, vitamin B-6, or vitamin B-12), determined from a detailed vitamin and mineral question asked on the HHHQ. Because the latter was related primarily to multivitamin use, supplemental folic acid, vitamin B-6, and vitamin B-12 were all highly correlated ($r >$ 0.90). Hence, separate variables for the individual supplements were not used in the analyses.

Blood collection and biochemical determinations

During the follow-up visit, venous blood samples were drawn from study participants after they had fasted for 12 h. Samples were collected in serum separator tubes, allowed to clot for 30 min, centrifuged for 15 min at 2000 × *g* at room temperature, and aliquoted into polypropylene tubes. One aliquot of serum was stored at −70 °C until analyzed and another aliquot was sent to a commercial laboratory for routine blood tests that included creatinine measurement.

In 1996 the stored samples were sent on dry ice to the Vitamin Metabolism Laboratory at the Jean Mayer US Department of Agriculture Human Nutrition Research Center at Tufts University. tHcy, expressed in μmol/L, was measured by using HPLC with fluorescence detection as described by Araki and Sako (30). The interassay CV from pooled plasma samples in this laboratory for homocysteine was 8%.

Other data

Body mass index (BMI; in kg/m^2) was calculated by using the weight and height measurements from the follow-up visit. Race-ethnicity was categorized as white or other. Estrogen replacement therapy and cigarette smoking habits were ascertained from a questionnaire and defined by current use (yes or no). Time from randomization to in-person follow-up visit was measured in days.

Analyses

Of the 297 participants in the VITAL pilot study, 260 were included in these analyses. Of the remaining 37 persons, 5 persons did not attend the follow-up visit; 1 person did not complete the HHHQ; 22 persons had unusable HHHQs, primarily because of the number of skipped questions; 1 woman had an extremely low energy intake (defined as <119 kJ for women and <143 kJ for men); and 8 persons had missing data (1 for tHcy, 1 for creatinine, 4 for age, and 2 for race). In addition to analyses of the full cohort (n = 260), analyses stratified by provision of the standard multivitamin [standard multivitamin given (n = 221) and standard multivitamin not given (n = 39)] were also performed.

The relation between tHcy and each nutrient was determined by using multivariable linear regression analyses in which ln-transformed homocysteine was the outcome variable. Independent variables included the well-accepted correlates of tHcy (dietary and prestudy supplemental folate and vitamin B-6, prestudy supplemental B-12, creatinine, sex, and age), putative determinants (coffee; percentage of energy from alcohol, protein, carbohydrate, and fat; dietary riboflavin; dietary protein as a proxy for methionine intake; smoking status; and estrogen use), and other variables potentially associated with tHcy (BMI, ethnicity, and duration of pill taking). Multivariable models were constructed with those variables significantly associated in bivariable models. Pearson correlations and plots of the variables were used to examine variable collinearity. Correlated variables were included in the same linear regression models to determine which variable more strongly predicted ln tHcy concentration. To illustrate the effects on tHcy of the significant categorical nutrient variables adjusted for other factors, generalized linear models were used to estimate the geometric means of tHcy concentrations. All statistical analyses were performed with SAS software (SAS Institute Inc, Cary, NC).

Analyses were performed separately for nutrients from foods and supplements and as continuous and categorical variables. For each nutrient, subjects were categorized by quintiles of intake according to the distribution of the nutrient in the VITAL population. Categories of coffee consumption were defined by the average number of cups consumed per day: nondrinkers (26% of the population), 1 cup (22%), 2 cups (23%), and 3–9 cups (29%). Trends of categorical variables were tested by creating a variable based on the median values of each category and using it in the linear regression models. Because duration of pill taking was not normally distributed, time was categorized by quintiles and a trend variable. Our analyses assumed that the study participants did not change their eating habits during the study.

Because protein, folate, riboflavin, and vitamin B-6 from foods were highly correlated with energy, they were adjusted for energy by using the residual method described by Willett and Stampfer (31). Log-transformed dietary nutrients were used for the energy adjustment. For examination of these nutrients in categories, energy was included in the multivariable models as a continuous, categorical, or trend variable.

Effect modification of the variables was determined by the addition of interaction terms to the multivariable models and stratification. Ninety-five percent CIs were determined for all effect estimates. SEs were determined for means. A P value <0.05 was considered significant.

TABLE 1
Characteristics of study participants[1]

Characteristic	Median or percentage
Female (%)	58
Age (y)	64 (60–69)[2]
Body mass index (kg/m^2)	
Female	26.2 (22.7–30.5)
Male	27.0 (24.3–30.2)
White (%)	86
Estrogen replacement users (% of women)	41 [62][3]
Current smokers (%)[4]	5 [12]
Standard multivitamin given (%)[5]	85 [221]
Duration of pill taking (d)[6]	88 (75–103)
Laboratory values (after intervention)	
Total serum homocysteine (μmol/L)	10.16 (8.21–12.93)
Serum creatinine (μmol/L)	88.4 (79.6–97.2)
Baseline daily dietary intake[7]	
Energy (kJ)	6483 (4768–7721)
Protein (% of energy)	17 (15–19)
Carbohydrate (% of energy)	48 (42–55)
Fat (% of energy)	33 (27–61)
Protein (g)	60 (45–76)
Folate (μg)	294 (217–374)
Vitamin B-6 (mg)	1.60 (1.20–2.02)
Riboflavin (mg)	1.68 (1.20–2.28)
Alcohol (% of energy)	<1 (0–4)
Coffee (cups/d)	2 (0–3)
Prestudy use of supplemental B vitamins (%)[8]	38

[1] n = 260.
[2] 25th to 75th percentile in parentheses.
[3] n in brackets.
[4] 25th to 75th percentile for number of cigarettes smoked per day: 10–20.
[5] Centrum (Lederle, Pearl River, NY) contains 400 μg folic acid, 6 μg vitamin B-12, and 2 mg vitamin B-6 per pill.
[6] Range: 46–133 d.
[7] Except for coffee, all nutrients and percentages of energy from protein, carbohydrate, fat, and alcohol were estimated from the Health Habits and History Questionnaire (29).
[8] Use of supplemental folic acid, vitamin B-6, or vitamin B-12. Range of nutrients: folic acid, 114–2000 μg; vitamin B-12, 1.7–36 μg; vitamin B-6, 0.6–15 mg. 25th to 75th percentiles were as follows: folic acid, 400–400 μg; vitamin B-12, 6–6 μg; vitamin B-6, 2–2 mg.

RESULTS

Characteristics of the study participants are shown in **Table 1**. The median serum tHcy concentration was 10.16 μmol/L (range: 2.81–33.16 μmol/L). The median age was 64 y. Most of the participants were white and female. Before enrollment, 38% of the participants consumed supplemental B vitamins (folic acid, vitamin B-12, and vitamin B-6), primarily in multivitamins.

Results of the multiple linear regression models in which nutrients and foods were entered as continuous variables are shown in **Table 2**. In the full cohort, coffee consumption was significantly and positively associated with ln tHcy, whereas baseline energy-adjusted dietary protein and prestudy supplemental B vitamin use were significantly and inversely associated with ln tHcy. Results were similar in parallel analyses restricted to the 221 persons who requested the standard multivitamin. BMI; smoking status; age; race-ethnicity; percentage of energy from alcohol, carbohydrate, and fat; dietary folate and vitamin B-6; randomization assignment (antioxidant or placebo); and receipt of the standard multivitamin (full cohort) did not significantly predict ln tHcy in any models. Percentage of energy from protein was significantly and inversely related to ln tHcy in models that also included percentage of energy from carbohydrate and fat and in models in which the other significant predicting variables were controlled for (not shown). The trend variable for categorical energy was positively related to ln tHcy in the full cohort with categorical dietary protein (P < 0.05) and in both the linear regression and generalized linear full models (P < 0.05).

There was no interaction by sex or other variables in any of the models, except between energy-adjusted dietary folate and standard multivitamin status (P = 0.04). In stratified analyses (Table 2), energy-adjusted dietary folate was nonsignificantly and inversely associated with ln tHcy among those who did not request the standard multivitamin and was nonsignificantly but positively associated with ln tHcy among those who requested the standard multivitamin. The time trend variable for duration of pill taking was not significantly associated with ln tHcy in any model, did not significantly interact with the variables included in the models or with standard multivitamin status, and did not significantly change the results when added to the full models that contained all of the significantly associated variables.

The dose-response effects of dietary protein and coffee from the results of the multivariable linear regression and generalized linear models for the entire cohort are shown in **Table 3** and **Figures 2** and **3**. In the analyses examining categorical protein, both adjusted and not adjusted for energy (not shown), the trend variable across quintiles and the fifth quintile of protein intake were significantly and inversely associated with ln tHcy in the full cohort and in those given the standard multivitamin. The predominant sources of dietary protein among the VITAL participants were low-fat animal foods (**Table 4**). The effect became stronger and more significant when energy was controlled for. Consumption of ≥3 cups coffee/d and the coffee trend variable were significantly related to greater ln tHcy concentration in the full cohort and in those not given the standard multivitamin.

Energy-adjusted dietary riboflavin predicted ln tHcy in the single-variable model for the full cohort and for those not given the standard multivitamin and was highly correlated with energy-adjusted dietary protein (r = 0.72, P = 0.0001; data not shown). However, when energy-adjusted riboflavin was included with energy-adjusted dietary protein in the multivariable models, it did not significantly predict ln tHcy and the effects of dietary protein remained significant. Energy-adjusted dietary folate and vitamin B-6 were less strongly correlated with energy-adjusted dietary protein (r = 0.41, P = 0.0001, and r = 0.40, P = 0.0001, respectively). Inclusion of these variables in the multivariable regression models did not change the effects of energy-adjusted dietary protein. The only exception was among those not given the standard multivitamin, in which case energy-adjusted folate increased the inverse association of energy-adjusted dietary protein with ln tHcy. In the single-variable model, female sex significantly and inversely predicted ln tHcy in the full cohort and among those not given the standard multivitamin (P < 0.01); these effects became nonsignificant, however, when creatinine was added to the models.

In separate regression analyses adjusted for the other covariates and restricted to the 151 female participants, estrogen replacement therapy was nonsignificantly and inversely related to ln tHcy for all models (full cohort: β = −0.0981, P = 0.10). In this subanalysis, the effects of serum creatinine, coffee, energy-

TABLE 2
Correlates of ln-transformed total serum homocysteine concentration: results from multiple linear regression analysis[1]

Independent variables	Full cohort (n = 260)		Standard multivitamin given (n = 221)		Standard multivitamin not given (n = 39)		
	β coefficient	P	β coefficient	P	β-coefficient	P	P for interaction
Dietary protein[2]	−0.3204 (−0.5405, −0.1003)[3]	0.005	−0.2828 (−0.5178, −0.0478)	0.02	−0.4683 (−1.1378, 0.2012)	0.18	0.15
Coffee (cups/d)	0.0373 (0.0124, 0.0622)	0.004	0.0310 (0.0043, 0.0578)	0.02	0.0862 (0.0194, 0.1530)	0.02	0.20
Prestudy use of supplemental B vitamins	−0.0997 (−0.1861, −0.0133)	0.03	−0.0977 (−0.1886, −0.0068)	0.04	0.1087 (−0.3519, 0.5693)	0.67	0.32
Serum creatinine (mmol/L)	6.1244 (3.4988, 8.7500)	0.0001	5.9785 (3.0995, 8.8574)	0.0001	5.6583 (−1.1662, 12.4830)	0.11	0.94
Dietary folate[4]	0.0275 (−0.1060, 0.1610)	0.68	0.0830 (−0.0632, 0.2292)	0.27	−0.1886 (−0.5173, 0.1401)	0.26	0.04
Intercept	1.7375[5]	—	1.7505[6]	—	1.7272[7]	—	—

[1] The models were controlled for all of the variables in the table. β coefficients are the ln of total serum homocysteine per unit change in the independent variable.
[2] ln of dietary protein adjusted for dietary energy by using the residual method.
[3] 95% CI in parentheses.
[4] ln of dietary folate adjusted for dietary energy by using the residual method.
[5] Adjusted R^2 = 0.12.
[6] Adjusted R^2 = 0.10.
[7] Adjusted R^2 = 0.23.

adjusted dietary protein, and prestudy use of supplemental B vitamins remained similarly associated with ln tHcy.

DISCUSSION

This study showed significant effects of dietary protein intake and coffee consumption on tHcy concentrations in older persons. For each of these dietary factors, dose-response relations were present such that increased dietary protein intake was associated with lower fasting tHcy concentrations and greater coffee consumption with higher fasting tHcy concentrations. These results were independent of known determinants of tHcy and were present in a variety of analyses. For each variable, the highest category of consumption was also significantly associated with tHcy.

The mechanism behind the inverse relation between protein intake and fasting tHcy concentrations is speculative. Dietary

TABLE 3
Multivariable linear regression models assessing the dose-response relation of protein and coffee with ln-transformed total serum homocysteine concentration[1]

Variables	Full cohort (n = 260)		Standard multivitamin given (n = 221)		Standard multivitamin not given (n = 39)	
	β coefficient	P	β coefficient	P	β coefficient	P
Protein quintile[2]						
1: 22.4–42.2 g/d	Reference [51]	—	Reference [44]	—	Reference [7]	—
2: 42.3–54.1 g/d	0.3837 (−1.0590, 1.8270) [52]	0.60	0.3469 (−1.1860, 1.8800) [46]	0.66	0.6223 (−3.7080, 4.9520) [6]	0.78
3: 54.3–66.0 g/d	−0.8534 (−2.5390, 0.8330) [52]	0.32	−0.7768 (−2.5860, 1.0320) [42]	0.40	0.3168 (−4.5480, 5.1820) [10]	0.89
4: 66.4–81.8 g/d	−1.5576 (−3.4810, 0.3650) [52]	0.11	−1.5778 (−3.6580, 0.5020) [42]	0.71	0.3142 (−5.0720, 5.7000) [10]	0.91
5: 81.9–171.8 g/d	−3.1055 (−5.3930, −0.8190) [53]	0.008	−2.5294 (−5.0180, −0.0400) [47]	0.05	−3.2381 (−9.7820, 3.3060) [6]	0.34
Coffee category[3]						
1: 0 cups/d	Reference [67]	—	Reference [56]	—	Reference [11]	—
2: 1 cup/d	−0.0019 (−0.1262, 0.1224) [56]	0.98	0.0130 (−0.1224, 0.1484) [47]	0.84	−0.1339 (−0.4630, 0.1952) [9]	0.43
3: 2 cups/d	0.0340 (−0.0867, 0.1547) [61]	0.58	0.0299 (−0.1014, 0.1612) [52]	0.64	0.1116 (−0.2147, 0.4379) [9]	0.50
4: 3–9 cups/d	0.1300 (0.0148, 0.2452) [76]	0.03	0.1069 (−0.0176, 0.2314) [66]	0.09	0.3290 (0.0157, 0.6424) [10]	0.05
Intercept	1.6171[4]	—	1.7454[5]	—	1.6721[6]	—

[1] n in brackets. 95% CI in parentheses. β coefficients represent the change in the ln of homocysteine for each independent variable or category.
[2] Each quintile of dietary protein was compared with the lowest quintile, adjusted for all other variables in the table, prestudy use of supplemental B vitamins, ln dietary folate adjusted for dietary energy by using the residual method, serum creatinine, and dietary energy as a trend variable. β coefficient represents the change in log homocysteine per 10 g protein. P values for the trend were 0.002, 0.02, and 0.17 in the models for the full cohort, standard multivitamin given, and standard multivitamin not given, respectively. P for the interaction was 0.71.
[3] Each category of coffee consumption was compared with the 0-cups/d category, adjusted for all other variables in the table, prestudy use of supplemental B vitamins, ln dietary folate adjusted for dietary energy by using the residual method, serum creatinine, and dietary energy as a trend variable. P values for the trend were 0.01, 0.07, and 0.02 in the models for the full cohort, standard multivitamin given, and standard multivitamin not given, respectively. P for the interaction was 0.12.
[4] Adjusted R^2 = 0.11.
[5] Adjusted R^2 = 0.09.
[6] Adjusted R^2 = 0.26.

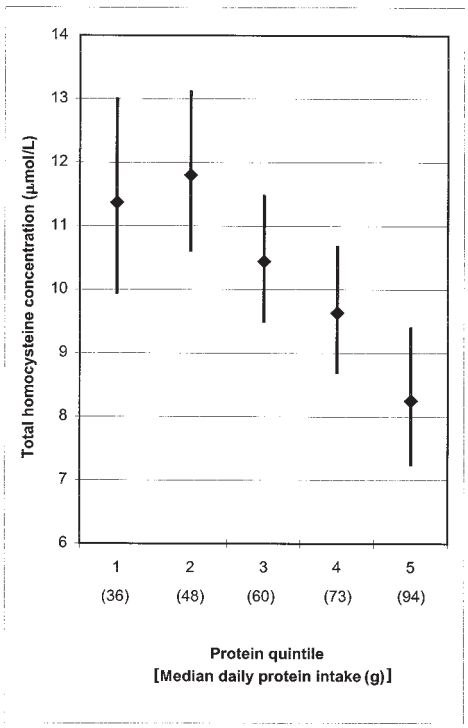

FIGURE 2. Geometric means (with 95% CIs) of serum total homocysteine (tHcy) concentrations for the full cohort (n = 260), by quintile of protein intake. Values are the antilog of general linear model procedure least-squares means of logarithmic tHcy concentration, adjusted for serum creatinine, coffee (continuous), supplemental folic acid, and dietary energy as a trend variable.

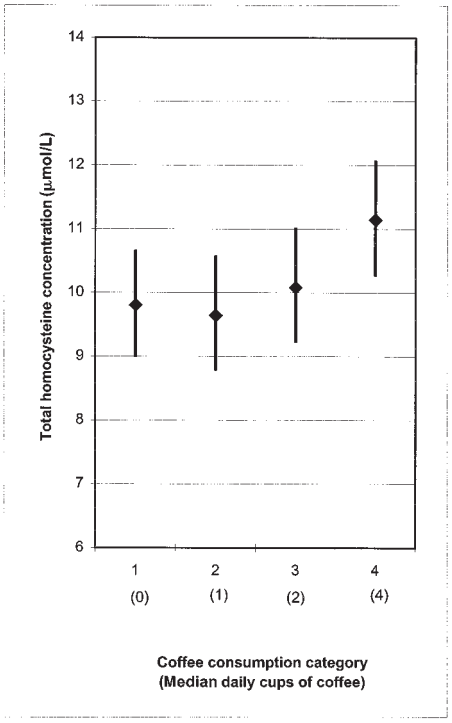

FIGURE 3. Geometric means (with 95% CIs) of serum total homocysteine (tHcy) concentrations for the full cohort (n = 260), by category of coffee consumption. Values are the antilog of general linear model procedure least-squares means of logarithmic tHcy concentration, adjusted for serum creatinine, energy-adjusted dietary protein (continuous), and supplemental folic acid.

methionine is correlated with dietary protein (32). Because oral methionine loading increases tHcy concentrations (11), we initially hypothesized that dietary protein would be positively associated with tHcy. Note that methionine loading represents a short-term, extreme situation, however, in which one amino acid, methionine, is metabolized through the homocysteine pathway (Figure 1). In contrast, protein intake, as examined in this study, represents long-term consumption. Short- and long-term changes in protein intake can alter protein catabolism (33). In addition, high-protein foods contain other amino acids and nutrients that could influence tHcy.

In animals, a high methionine intake induces more efficient catabolism of homocysteine through activation of homocysteine-catabolizing enzymes. It has been shown that the transsulfuration (Figure 1, reaction number 4) and, to a lesser extent, the methionine regeneration (Figure 1, reaction number 3) pathways are activated in the livers of animals fed excessive amounts of methionine (14, 34–36). Finkelstein and Martin (34) believed that serine and betaine were the limiting factors for their respective reactions with excess methionine. Andersson et al (37) found no changes in results of a postmethionine load test, methionine clearance, or tHcy concentration after excess methionine was added to 6 human subjects' usual diets for 13 d. This study, however, had limited power because of small numbers, did not control for differences in the participants' usual diets or energy intake, and limited the feeding of the additional methionine to the 2 wk before the test (37).

High protein intakes might have beneficial physiologic effects (38–43). Preagricultural humans evolved on a diet high in animal protein (37% protein), low in fat (22%), and high in fruit and vegetables (41% carbohydrate) (44). Recently, in the Nurses' Health Study (45), higher methionine intake was prospectively associated with less coronary artery disease (relative risk: 0.82; 95% CI: 0.65, 1.03), independent of dietary folate, other cardiovascular risk factors, and vitamin B-12 intake (relative risk: 1.09; 95% CI: 0.82, 1.44). If high-protein diets are not limited in serine or choline, it is biologically plausible that these 2 pathways could be increasing tHcy clearance from the blood and possibly increasing survival.

TABLE 4
Top food protein sources in the Vitamin, Teachers, and Longevity (VITAL) population

Rank and food source	Percentage of total
	%
1: Skim milk	9.9
2: Chicken or turkey without skin	7.4
3: 2% Milk	5.4
4: Spaghetti	4.9
5: Beef (with fat trimmed off)	4.5
6: Cheese and cheese spread	3.6
7: White bread and rolls	3.3
8: Dark bread	3.2
9: Fish, broiled or baked	3.0
10: Eggs	2.2
11: Hamburger, beef burrito, meatloaf	2.1
Cumulative total	49.5

An alternative explanation is that vitamin B-12 accounts for the apparent association between protein and tHcy. The predominant sources of protein in our population, namely animal foods (Table 4), are also sources of vitamin B-12. Milk, which has been inversely associated with tHcy (13), is often consumed with vitamin-fortified cold breakfast cereals that contain crystalline vitamin B-12, a more absorbable form of vitamin B-12 also found in vitamin supplements. Those who consume these foods for extended periods of time could have greater vitamin B-12 stores and be protected from later development of marginal vitamin B-12 status and elevated tHcy concentrations. Dietary vitamin B-12, however, is not necessarily correlated with vitamin B-12 status in older persons (12, 13, 46) and the oral dose of supplemental vitamin B-12 required to maintain adequate status with malabsorption, the most common cause of vitamin B-12 deficiency in the elderly (46, 47), may be greater than that in typical multivitamins or in the diet (48, 49). In contrast, persons with atrophic gastritis, which accounts for a substantial proportion of adults with vitamin B-12 deficiency (50), can absorb crystalline vitamin B-12 normally (51). In the Framingham Offspring Study (n = 2999 subjects aged 26–83 y), subjects who took vitamin supplements or ate fortified breakfast cereals were less likely to have low vitamin B-12 status than those who did not take vitamin supplements or eat fortified breakfast cereals (52). Two studies, however, that examined associations between dietary or supplemental vitamin B-12 and tHcy in the older Framingham population found no association or a weak correlation, respectively (12, 48).

The positive, independent association and dose-response relation between tHcy and coffee consumption (Table 3 and Figure 3) is consistent with one other study (28). Although the association may be real, its biological basis is unclear. Nygård et al (28) showed a significant dose-response relation for caffeinated coffee but not decaffeinated coffee or caffeinated tea in a Norwegian population (n = 16 175). Caffeinated tea contains moderate amounts of folate and less caffeine than caffeinated coffee. The authors postulated that these factors could explain their findings. In contrast, Nieto et al (53) found no significant association between coffee consumption or caffeine and tHcy in a sample from the Atherosclerosis Risk in Communities (ARIC) study (n = 537). The ARIC sample however, had a smaller proportion of heavy coffee drinkers (>3 cups/d: ≈16% compared with >38%) and a smaller sample size than did the study by Nygård et al. Alternatively, the apparent association in the study by Nygård et al and in our study may have resulted from uncontrolled or residual confounding. The primary confounder of coffee consumption and disease in other studies, namely, current smoking (54), was not an issue in this study because only 12 participants were smokers. Coffee drinkers could have other lifestyle characteristics that influence tHcy concentrations. If coffee consumption raises tHcy, such a relation may provide an explanation by which high coffee consumption increases the risk of vascular disease.

The fact that our study replicated relations of tHcy shown in other studies enhances the validity of our results. We documented a significant association between serum creatinine and ln tHcy, as shown in other studies (11, 24, 26). We also observed a modest, nonsignificant inverse association between estrogen replacement therapy and ln tHcy among women. Other studies have reported a significant inverse association between estrogen replacement therapy and tHcy (55, 56). The lack of significance in our study likely resulted from the small number of women in the analysis. The lack of a significant association between age and tHcy in any of our models was likely due to the narrow age range of our study participants. Finally, 3 studies showed less striking inverse relations between dietary protein and tHcy (13, 57, 58). Our more impressive relation may have been due to the wide range of protein intake, which varied threefold among participants.

In the full cohort, prestudy use of supplemental B vitamins (folic acid, vitamin B-6, and vitamin B-12) was associated with ln tHcy; in contrast, provision of the standard multivitamin (given to 85% of study participants) was not associated with ln tHcy. The facts that most participants requested the standard multivitamin and that the combined dietary and multivitamin folate intake provided >600 μg folate at the time serum was drawn (at the end of the intervention) may explain why provision of the standard multivitamin did not significantly predict tHcy in the full cohort (12, 55, 59). In addition, a flat relation between folate and tHcy at high folate concentrations could have accounted for the significant interaction between the provision of the standard multivitamin and dietary folate (Table 2), as well as the small R^2 in the full cohort model and in the model that included participants who were given the standard multivitamin. Also, the effects of prestudy use of supplemental B vitamins represented long-term exposure whereas the standard multivitamin represented brief and recent supplementation. Finally, persons who are long-term supplement users may have other habits or characteristics that could influence tHcy status (eg, avoiding environmental cigarette smoke and a healthier lifestyle).

Potential limitations of our study include misclassification of exposures and the extent to which our findings can be generalized to other populations. The results from our study may pertain only to older persons.

In conclusion, we found a strong inverse relation between protein intake and serum ln tHcy concentrations in older persons. In addition, we found independent positive relations between ln tHcy and coffee and serum creatinine and an inverse association between ln tHcy and prestudy use of supplemental B vitamins. Additional studies, particularly clinical trials, will be required to determine whether the observed associations reflect cause-effect relations.

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