

# Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort<sup>1-4</sup>

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## ABSTRACT

**Background:** Established determinants of fasting total homocysteine (tHcy) concentration include folate and vitamin B-12 status, serum creatinine concentration, and renal function.

**Objective:** Our objective was to examine the relation between known and suspected determinants of fasting plasma tHcy in a population-based cohort.

**Design:** We examined the relations between fasting plasma tHcy concentrations and nutritional and other health factors in 1960 men and women, aged 28–82 y, from the fifth examination cycle of the Framingham Offspring Study between 1991 and 1994, before the implementation of folic acid fortification.

**Results:** Geometric mean tHcy was 11% higher in men than in women and 23% higher in persons aged  $\geq 65$  y than in persons aged  $< 45$  y ( $P < 0.001$ ). tHcy was associated with plasma folate, vitamin B-12, and pyridoxal phosphate ( $P$  for trend  $< 0.001$ ). Dietary folate, vitamin B-6, and riboflavin were associated with tHcy among non-supplement users ( $P$  for trend  $< 0.01$ ). The tHcy concentrations of persons who used vitamin B supplements were 18% lower than those of persons who did not ( $P < 0.001$ ). tHcy was positively associated with alcohol intake ( $P$  for trend = 0.004), caffeine intake ( $P$  for trend  $< 0.001$ ), serum creatinine ( $P$  for trend  $< 0.001$ ), number of cigarettes smoked ( $P$  for trend  $< 0.001$ ), and antihypertensive medication use ( $P < 0.001$ ).

**Conclusions:** Our study confirmed, in a population-based setting, the importance of the known determinants of fasting tHcy and suggested that other dietary and lifestyle factors, including vitamin B-6, riboflavin, alcohol, and caffeine intakes as well as smoking and hypertension, influence circulating tHcy concentrations. *Am J Clin Nutr* 2001;73:613–21.

**KEY WORDS** Homocysteine, diet, folate, vitamin B-12, creatinine, smoking, caffeine, alcohol consumption, hypertension, epidemiology

## INTRODUCTION

Homocysteine, a non-protein-forming sulfur amino acid, is an intermediate in methionine metabolism. Elevated circulating homocysteine concentration is associated with an increased risk of occlusive vascular disease (1, 2). Established determinants of fasting total homocysteine (tHcy) concentration include folate and vitamin B-12 status, serum creatinine concentration, and renal function. Elevated fasting tHcy concentrations are associated with lower circulating concentrations or intakes of folate

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and vitamin B-12 (1–4) and are amenable to treatment with these vitamins (5–9). The relation between circulating concentrations of tHcy and serum creatinine (10–12) may largely reflect the effect of renal function on homocysteine concentrations, but tHcy and creatinine could also be related because of increased homocysteine production during creatine metabolism (10). Renal failure is accompanied by elevated tHcy concentrations (13) and there is a strong inverse relation between directly measured glomerular filtration rate and tHcy concentrations across the entire range of renal function (14, 15). However, normal urinary clearance of homocysteine is very low and the importance of renal uptake and metabolism of homocysteine in homocysteine elimination remains controversial (13).

Several other nutritional factors may contribute to higher fasting tHcy concentrations. Riboflavin is a cofactor for methylenetetrahydrofolate reductase, an enzyme involved in the remethylation of homocysteine to methionine (16). Vitamin B-6 plays a role in homocysteine transsulfuration and catabolism, but the relation between vitamin B-6 status and fasting tHcy concentrations has received little attention. An inverse association was reported between circulating tHcy concentrations and protein intake (17), and positive associations were found between homocysteine and consumption of alcohol (18) and coffee (17, 19). We used data on nutrition, health, and lifestyle from the fifth examination cycle of the Framingham Offspring cohort to examine known and suspected determinants of fasting plasma tHcy concentrations.

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## SUBJECTS AND METHODS

### Study subjects

The Framingham Heart Study, an epidemiologic study of heart disease, was established in Framingham, MA, between 1948 and 1950 with a cohort of 5209 men and women aged 30–59 y (20). By 1971, the original cohort included 1644 husband-wife pairs and 378 individuals who had developed cardiovascular disease. The offspring of these subjects and the offsprings' spouses were invited to participate in the first Framingham Offspring Study, and 5135 of the 6838 eligible individuals participated examination (21). The Framingham Offspring cohort has undergone repeat examinations at  $\approx 3$ –4 y intervals. The fifth examination of the offspring cohort began in January 1991 and was completed in December 1994. This study was approved by the Human Investigations Review Committee at New England Medical Center and by the Institutional Review Board for Human Research at Boston University Medical Center.

### Measurements

As part of the fifth offspring cohort examination, fasting (>10 h) blood samples were obtained for determination of homocysteine, folate, vitamin B-12, and pyridoxal-5'-phosphate (PLP; the active circulating form of vitamin B-6). Plasma tHcy was measured by HPLC with fluorometric detection (22), plasma folate by a 96-well plate microbial (*Lactobacillus casei*) assay (23, 24), plasma PLP by the tyrosine decarboxylase apoenzyme method (25), and plasma vitamin B-12 by a radioassay (Quantaphase II; Bio-Rad, Hercules, CA). The CVs for these assays were 8% for tHcy, 13% for folate, 16% for PLP, and 7% for vitamin B-12.

Usual dietary intakes of folate, vitamin B-12, vitamin B-6, and riboflavin were assessed with a food-frequency questionnaire (26). The food-frequency questionnaire also identifies nutrient intake from dietary supplements and from fortified, ready-to-eat breakfast cereals.

Of the 3799 individuals who attended the fifth examination cycle of the Framingham Offspring Study, 1960 had valid food-frequency questionnaires; complete data on tHcy, vitamin, and creatinine concentrations; were free of diagnosed cardiovascular disease; and were not taking medications that might alter tHcy concentrations. These 920 men and 1040 women comprised the sample for our analyses.

### Statistical analyses

Because plasma tHcy concentration was positively skewed, analyses were done by using natural logarithmic transformations. Inverse transformations were performed to provide geometric means and their 95% CIs.

We determined the age- and sex-adjusted and multivariate-adjusted geometric mean tHcy concentrations and 95% CIs within categories of its known and suspected determinants using SAS PROC GLM (27). To estimate mean tHcy concentration across levels of established and suspected tHcy determinants, continuous variables were divided into categories for analysis. Categories for most variables were based on quintile cutoff points. The exceptions were age, number of cigarette smoked per day, alcohol intake, and beverage consumption. Individuals who consumed, on a daily basis, two-thirds of the recommended dietary allowance (RDA) of folate, vitamin B-12, vitamin B-6, or riboflavin (28) in the form of supplements were designated as

regular users of vitamin B supplements. The analyses of relations between tHcy and dietary vitamins excluded individuals who were regular users of vitamin B supplements, and analyses of the relations between tHcy and blood pressure excluded individuals who reported use of blood pressure-lowering medications. We also present the *P* value for the comparison of each category of a variable with a reference category, except when the *F* statistic from the analysis of variance was not significant.

In addition to terms for age and sex, the multivariate models included serum creatinine concentrations and plasma folate, vitamin B-12, and PLP concentrations with the following exceptions. For the analyses of use of vitamin B supplements and nutrient intake, including folate, vitamin B-12, vitamin B-6, riboflavin, protein, and methionine intake, the adjustment included dietary intake of folate, vitamin B-12, and vitamin B-6 in place of the corresponding plasma vitamins.

Tests for trend, which were performed for the continuous variables, were based on the significance of the linear regression coefficient relating the variable in its continuous form to the logarithm of plasma tHcy. The continuous variables were transformed as needed so that they were linearly related to the logarithm of tHcy. A logarithmic transformation was applied to serum creatinine; plasma concentrations of folate, vitamin B-12, and PLP; and dietary intakes of riboflavin, vitamin B-6, and vitamin B-12, and alcohol. A square root transformation was applied to dietary folate intake.

Final multivariate models, one based on the plasma vitamin information and one based on the dietary information, were determined by using a backward selection procedure for all factors that were significantly associated with tHcy concentrations after the previously described multivariate adjustment procedure. The continuous variables were entered into these models with use of the appropriate transformations as described previously.

## RESULTS

The mean age was 54 y for both men and women (range: 28–82 y). The geometric mean tHcy concentrations by age group, sex, and other potential nonnutrient determinants of tHcy concentrations are shown in **Table 1**. The geometric mean was 11% higher in men than in women and 23% higher in persons aged  $\geq 65$  y than in those aged <45 y after adjustment for known determinants of circulating tHcy concentration. Serum creatinine displayed a strong, positive association with tHcy concentrations. Geometric mean tHcy concentrations were 20% higher in the highest serum creatinine quintile category than in the lowest quintile category ( $P < 0.004$ ). Body mass index (BMI; in  $\text{kg}/\text{m}^2$ ) showed a weak positive association with circulating tHcy ( $P = 0.03$ ). Persons who smoked  $\geq 26$  cigarettes/d had tHcy concentrations that were 16% higher than those of nonsmokers ( $P < 0.001$ ) and the tHcy concentrations of persons who were using antihypertensive medications were 9% higher than were those of persons who were not using such medications ( $P < 0.001$ ). Neither systolic nor diastolic blood pressure was related to tHcy concentrations in persons who were not using antihypertensive medications.

The relation between plasma tHcy concentration and plasma vitamin B concentrations is shown in **Table 2**. After multivariate adjustment, geometric mean tHcy concentration was 49% higher in the lowest than in the highest quintile category of plasma folate ( $P$  for trend < 0.001). More modest (17% and 12%) differences between the lowest and highest categories

**TABLE 1**

Geometric mean total plasma homocysteine by age, sex, and categories of its established and suspected nondietary determinants

Variable	Total plasma homocysteine			
	Age and sex adjusted	<i>P</i>	Multivariable adjusted <sup>1</sup>	<i>P</i>
	$\mu\text{mol/L}$		$\mu\text{mol/L}$	
<b>Sex</b>				
Female ( <i>n</i> = 1040) <sup>2</sup>	8.8 (8.6, 8.9) <sup>3</sup>		9.0 (8.8, 9.2)	
Male ( <i>n</i> = 920)	10.3 (10.1, 10.5)	<0.001	10.0 (9.8, 10.2)	<0.001
<b>Age (y)</b>				
<45 ( <i>n</i> = 324) <sup>2</sup>	8.8 (8.5, 9.1)		8.6 (8.3, 8.8)	
45–54 ( <i>n</i> = 701)	9.2 (9.0, 9.4)	0.05	9.1 (8.9, 9.3)	<0.001
55–64 ( <i>n</i> = 617)	9.8 (9.6, 10.1)	<0.001	9.8 (9.6, 10.1)	<0.001
≥65 ( <i>n</i> = 318)	10.4 (10.0, 10.7)	<0.001	10.6 (10.3, 10.9)	<0.001
		<0.001 <sup>4</sup>		<0.001
<b>Body mass index (kg/m<sup>2</sup>)</b>				
<23.2 ( <i>n</i> = 390) <sup>2</sup>	9.4 (9.1, 9.7)	— <sup>5</sup>	9.4 (9.1, 9.6)	—
23.2–25.4 ( <i>n</i> = 391)	9.4 (9.1, 9.6)	—	9.3 (9.1, 9.6)	—
25.5–27.6 ( <i>n</i> = 391)	9.6 (9.3, 9.9)	—	9.6 (9.3, 9.9)	—
27.7–30.6 ( <i>n</i> = 391)	9.2 (9.0, 9.5)	—	9.4 (9.1, 9.6)	—
≥30.7 ( <i>n</i> = 391)	9.9 (9.6, 10.2)	—	9.7 (9.4, 10.0)	—
		0.02		0.03
<b>Serum creatinine (<math>\mu\text{mol/L}</math>)</b>				
<79 ( <i>n</i> = 364) <sup>2</sup>	8.7 (8.4, 9.0)		8.7 (8.5, 9.0)	
79–<87 ( <i>n</i> = 381)	9.3 (9.0, 9.6)	0.003	9.3 (9.0, 9.5)	0.003
87–<96 ( <i>n</i> = 423)	9.3 (9.0, 9.6)	0.002	9.3 (9.0, 9.5)	0.002
96–<106 ( <i>n</i> = 378)	9.7 (9.4, 10.0)	<0.001	9.6 (9.4, 9.9)	<0.001
≥106 ( <i>n</i> = 414)	10.5 (10.1, 10.8)	<0.001	10.4 (10.1, 10.7)	<0.001
		<0.001 <sup>4</sup>		0.004
<b>Current cigarette smoking (cigarettes/d)</b>				
0 ( <i>n</i> = 1582) <sup>2</sup>	9.3 (9.2, 9.5)		9.3 (9.2, 9.5)	
1–15 ( <i>n</i> = 118)	9.9 (9.3, 10.5)	0.05	9.8 (9.3, 10.3)	0.06
16–25 ( <i>n</i> = 141)	10.1 (9.6, 10.7)	0.003	9.8 (9.3, 10.2)	0.07
≥26 ( <i>n</i> = 117)	11.0 (10.4, 11.6)	<0.001	10.8 (10.3, 11.4)	<0.001
		<0.001 <sup>4</sup>		<0.001
<b>Systolic blood pressure (mm Hg)<sup>6</sup></b>				
<108 ( <i>n</i> = 307) <sup>2</sup>	9.1 (8.8, 9.5)	—	9.3 (9.0, 9.6)	—
108–116 ( <i>n</i> = 322)	9.4 (9.0, 9.7)	—	9.3 (9.0, 9.6)	—
117–125 ( <i>n</i> = 347)	9.1 (8.8, 9.4)	—	9.0 (8.8, 9.3)	—
126–136 ( <i>n</i> = 340)	9.4 (9.1, 9.8)	—	9.4 (9.1, 9.7)	—
≥137 ( <i>n</i> = 344)	9.5 (9.2, 9.9)	—	9.5 (9.2, 9.8)	—
		0.40 <sup>f</sup>		0.93
<b>Diastolic blood pressure (mm Hg)<sup>6</sup></b>				
<65 ( <i>n</i> = 322) <sup>2</sup>	9.4 (9.1, 9.7)	—	9.4 (9.1, 9.6)	—
66–70 ( <i>n</i> = 305)	9.0 (8.6, 9.3)	—	9.1 (8.8, 9.4)	—
71–75 ( <i>n</i> = 334)	9.5 (9.2, 9.8)	—	9.5 (9.2, 9.8)	—
76–81 ( <i>n</i> = 361)	9.2 (8.9, 9.5)	—	9.2 (8.9, 9.4)	—
≥82 ( <i>n</i> = 338)	9.5 (9.2, 9.9)	—	9.4 (9.1, 9.6)	—
		0.35		0.99
<b>Hypertensive medication use</b>				
No ( <i>n</i> = 1660) <sup>2</sup>	9.4 (9.2, 9.5)		9.4 (9.2, 9.5)	
Yes ( <i>n</i> = 296)	10.2 (9.8, 10.6)	<0.001 <sup>4</sup>	10.2 (9.8, 10.5)	<0.001

<sup>1</sup> Adjusted for age, sex, serum creatinine concentration, and plasma folate, vitamin B-12, and pyridoxal phosphate concentrations.<sup>2</sup> Reference category for statistical comparisons.<sup>3</sup>  $\bar{x}$  (95% CI).<sup>4</sup> *P* for trend for linear regression coefficient of continuous variable.<sup>5</sup> *F* statistic from analysis of variance was not significant (*P* > 0.05) and individual comparisons are not presented.<sup>6</sup> Blood pressure analyses are based on the subset of subjects who did not take medications that affect blood pressure.

were seen for vitamin B-12 (*P* for trend < 0.001) and PLP (*P* for trend < 0.001), respectively.

Dietary intakes of folate and vitamin B-6 were strongly associated with plasma tHcy concentrations among nonusers of supplements (*P* for trend < 0.001 for both), whereas dietary vitamin B-12 intake showed no consistent association with tHcy concentration after multivariate adjustment (**Table 3**). The

difference in geometric mean tHcy concentrations between the lowest and highest quintile categories was 16% for folate intake (*P* < 0.001) and 17% for vitamin B-6 intake (*P* < 0.001). Lower dietary riboflavin intake was also associated with a modestly higher tHcy concentration (10%; *P* < 0.01). tHcy concentrations were 18% lower in individuals who did use vitamin B supplements than in those who did not (*P* < 0.001) after

**TABLE 2**

Geometric mean total plasma homocysteine by categories of its established and suspected plasma vitamin determinants

Variable	Total plasma homocysteine			
	Age and sex adjusted	<i>P</i>	Multivariable adjusted <sup>1</sup>	<i>P</i>
	$\mu\text{mol/L}$		$\mu\text{mol/L}$	
Plasma folate (nmol/L)				
<6.9 ( <i>n</i> = 392)	11.9 (11.6, 12.3) <sup>2</sup>	<0.001	11.8 (11.5, 12.1)	<0.001
6.9–10.0 ( <i>n</i> = 392)	10.3 (10.0, 10.6)	<0.001	10.1 (9.9, 10.4)	<0.001
10.1–15.2 ( <i>n</i> = 392)	9.6 (9.3, 9.8)	<0.001	9.5 (9.3, 9.8)	<0.001
15.3–23.9 ( <i>n</i> = 391)	8.5 (8.3, 8.7)	<0.001	8.6 (8.3, 8.8)	<0.001
≥24.0 ( <i>n</i> = 393) <sup>3</sup>	7.7 (7.5, 7.9)	<0.001 <sup>4</sup>	7.9 (7.7, 8.1)	<0.001 <sup>4</sup>
Plasma vitamin B-12 (pmol/L)				
<197 ( <i>n</i> = 392)	10.9 (10.6, 11.2)	<0.001	10.5 (10.2, 10.8)	<0.001
197–262 ( <i>n</i> = 392)	9.8 (9.5, 10.1)	<0.001	9.6 (9.4, 9.9)	<0.001
263–330 ( <i>n</i> = 392)	9.5 (9.2, 9.7)	<0.001	9.3 (9.1, 9.6)	0.05
331–431 ( <i>n</i> = 392)	9.0 (8.7, 9.2)	0.04	9.1 (8.8, 9.3)	0.56
≥432 ( <i>n</i> = 392) <sup>3</sup>	8.6 (8.3, 8.8)	<0.001 <sup>4</sup>	9.0 (8.7, 9.2)	<0.001 <sup>4</sup>
Plasma pyridoxal phosphate (nmol/L)				
<38.2 ( <i>n</i> = 392)	11.0 (10.6, 11.3)	<0.001	10.3 (10.0, 10.6)	<0.001
38.3–50.6 ( <i>n</i> = 392)	9.9 (9.6, 10.2)	<0.001	9.5 (9.2, 9.8)	0.17
50.7–65.1 ( <i>n</i> = 391)	9.5 (9.2, 9.7)	<0.001	9.3 (9.1, 9.6)	0.66
65.2–92.4 ( <i>n</i> = 393)	8.9 (8.7, 9.2)	0.006	9.1 (8.9, 9.4)	0.62
≥92.5 ( <i>n</i> = 392) <sup>3</sup>	8.4 (8.2, 8.7)	<0.001 <sup>4</sup>	9.2 (9.0, 9.5)	<0.001 <sup>4</sup>

<sup>1</sup> Adjusted for age, sex, serum creatinine concentration, and plasma folate, vitamin B-12, and pyridoxal phosphate concentrations.<sup>2</sup>  $\bar{x}$  (95% CI).<sup>3</sup> Reference category for statistical comparisons.<sup>4</sup> *P* for trend for linear regression coefficient for continuous variable.

multivariate adjustment that included dietary intake of folate, vitamin B-12, and vitamin B-6.

There were strong inverse associations between protein and methionine intake and tHcy concentrations after adjustment for age and sex (Table 3). However, the adjustment for dietary vitamin B-6 intake, and to a lesser extent folate intake, resulted in the weakening or disappearance of the association between tHcy and both protein and methionine. After multivariate adjustment, there were no significant differences between the geometric mean tHcy concentrations between the lowest and highest intake categories for protein and methionine, but concentrations in the intermediate protein intake categories tended to be lower, and in some cases significantly lower, than concentrations in the lowest intake category.

Intakes of both alcohol and caffeine were positively associated with tHcy concentrations after adjustment for the other nutritional determinants of tHcy concentrations (Table 3). Modestly higher tHcy concentrations (<4%) were seen in individuals who consumed on average >15 g alcohol/d (*P* for trend = 0.004). Mean tHcy concentrations were significantly higher in the 4 highest caffeine quintile categories (≥89 mg/d) than in the lowest category (*P* for trend < 0.001).

The relation between consumption of caffeinated and alcoholic beverages and tHcy concentrations is shown in **Table 4**. Consumption of ≥1 cup of coffee or ≥2 cans of caffeinated cola/d was associated with higher tHcy concentrations. There was a weak inverse association between tea consumption and tHcy, which remained significant after adjustment for coffee consumption. Consumption of liquor was strongly associated

with higher tHcy concentrations. tHcy concentration was only weakly related to consumption of red wine and was not related to consumption of white wine or beer.

All of the variables in Tables 1–3 that were significantly associated with tHcy concentrations in the previous multivariate analyses were entered simultaneously into either a plasma vitamin or vitamin intake regression model to determine which variables remained associated with tHcy concentrations after mutual adjustment for each other. The plasma vitamin model included age; sex; plasma folate, vitamin B-12, and PLP concentrations; alcohol intake; caffeine intake; BMI; serum creatinine concentration; smoking status; and use of antihypertensive medications. All variables except PLP remained significantly associated with plasma tHcy concentrations in this model. The remaining variables in order of their significance were plasma folate, age, serum creatinine, plasma vitamin B-12, sex, treatment for hypertension, smoking, alcohol consumption, caffeine intake, and BMI. On further examination, it was determined that the inclusion of current cigarette smoking in the regression model was largely responsible for the removal of PLP from the model. The PLP concentration was 14.7 nmol/L lower in smokers than in nonsmokers after adjustment for vitamin B-6 intake (*P* < 0.001). All variables entered into the vitamin intake model remained significantly associated with plasma tHcy concentration, except for vitamin B-6 intake. The order of the variables from most to least significant was serum creatinine concentration, age, sex, folate intake, smoking status, riboflavin intake, treatment for hypertension, caffeine intake, alcohol intake, BMI, and use of vitamin B supplements. Further analyses showed that vitamin B-6 intake

**TABLE 3**

Geometric mean total plasma homocysteine by categories of its established and suspected dietary determinants

Variable	Total plasma homocysteine			
	Age and sex adjusted	<i>P</i>	Multivariable adjusted <sup>1</sup>	<i>P</i>
	$\mu\text{mol/L}$		$\mu\text{mol/L}$	
Dietary folate intake ( $\mu\text{g/d}$ ) <sup>2</sup>				
<191 ( <i>n</i> = 287)	11.6 (11.3, 12.0) <sup>3</sup>	<0.001	11.1 (10.6, 11.6)	<0.001
191–253 ( <i>n</i> = 287)	10.4 (10.0, 10.7)	<0.001	10.2 (9.8, 10.5)	0.08
254–313 ( <i>n</i> = 288)	9.8 (9.5, 10.1)	0.002	9.7 (9.4, 10.1)	0.64
314–388 ( <i>n</i> = 287)	9.5 (9.2, 9.8)	0.06	9.7 (9.3, 10.0)	0.85
$\geq 387$ ( <i>n</i> = 288) <sup>4</sup>	9.1 (8.8, 9.4)		9.6 (9.2, 10.0)	
		<0.001 <sup>5</sup>		<0.001 <sup>5</sup>
Dietary vitamin B-12 intake ( $\mu\text{g/d}$ ) <sup>2</sup>				
<3.05 ( <i>n</i> = 287)	10.8 (10.4, 11.2)	0.001	10.0 (9.6, 10.4)	0.08
3.05–4.27 ( <i>n</i> = 287)	10.2 (9.9, 10.6)	0.04	9.8 (9.5, 10.2)	0.02
4.28–5.41 ( <i>n</i> = 288)	10.1 (9.7, 10.4)	0.17	10.0 (9.7, 10.4)	0.06
5.42–7.56 ( <i>n</i> = 286)	9.5 (9.1, 9.8)	0.30	9.8 (9.5, 10.1)	0.002
$\geq 7.57$ ( <i>n</i> = 289) <sup>4</sup>	9.7 (9.4, 10.1)		10.6 (10.2, 11.0)	
		<0.001 <sup>5</sup>		0.06 <sup>5</sup>
Dietary vitamin B-6 intake ( $\text{mg/d}$ ) <sup>2</sup>				
<1.25 ( <i>n</i> = 285)	11.5 (11.0, 11.9)	<0.001	11.1 (10.6, 11.5)	<0.001
1.25–1.62 ( <i>n</i> = 289)	10.4 (10.0, 10.7)	<0.001	10.2 (9.8, 10.5)	0.05
1.63–1.96 ( <i>n</i> = 285)	10.0 (9.7, 10.3)	<0.001	9.9 (9.6, 10.2)	0.17
1.97–2.39 ( <i>n</i> = 289)	9.5 (9.2, 9.8)	0.03	9.6 (9.3, 9.9)	0.70
$\geq 2.40$ ( <i>n</i> = 289) <sup>4</sup>	9.0 (8.7, 9.3)		9.5 (9.1, 10.0)	
		<0.001 <sup>5</sup>		<0.001 <sup>5</sup>
Dietary riboflavin intake ( $\text{mg/d}$ ) <sup>2</sup>				
<1.11 ( <i>n</i> = 280)	11.4 (11.0, 11.8)	<0.001	10.8 (10.3, 11.2)	0.01
1.11–1.42 ( <i>n</i> = 286)	10.5 (10.1, 10.8)	<0.001	10.2 (9.8, 10.6)	0.23
1.43–1.74 ( <i>n</i> = 293)	9.8 (9.5, 10.1)	0.003	9.8 (9.4, 10.1)	0.84
1.75–2.20 ( <i>n</i> = 288)	9.6 (9.3, 9.9)	0.05	9.7 (9.4, 10.0)	0.68
$\geq 2.21$ ( <i>n</i> = 290) <sup>4</sup>	9.1 (8.8, 9.4)		9.8 (9.4, 10.3)	
		<0.001 <sup>5</sup>		0.003 <sup>5</sup>
Regular use of vitamin B supplements				
Yes ( <i>n</i> = 523) <sup>4</sup>	8.1 (7.9, 8.3)		8.2 (8.0, 8.4)	
No ( <i>n</i> = 1437)	10.1 (9.9, 10.2)	<0.001	10.0 (9.9, 10.1)	<0.001
Protein intake ( $\text{g/d}$ ) <sup>6</sup>				
<55.2 ( <i>n</i> = 392) <sup>4</sup>	10.4 (10.1, 10.7)		9.3 (9.0, 9.6)	
55.2–67.9 ( <i>n</i> = 392)	9.7 (9.4, 10.0)	0.002	9.0 (8.7, 9.2)	0.11
68.0–80.9 ( <i>n</i> = 392)	9.3 (9.1, 9.6)	<0.001	8.8 (8.5, 9.1)	0.02
81.0–97.6 ( <i>n</i> = 392)	9.0 (8.7, 9.3)	<0.001	8.9 (8.6, 9.2)	0.08
$\geq 97.7$ ( <i>n</i> = 392)	9.1 (8.8, 9.4)	<0.001	9.3 (9.0, 9.6)	0.86
		<0.001 <sup>5</sup>		0.11 <sup>5</sup>
Methionine intake ( $\text{g/d}$ ) <sup>6</sup>				
<1.24 ( <i>n</i> = 382) <sup>4</sup>	10.3 (10.0, 10.6)		9.3 (9.0, 9.6)	— <sup>7</sup>
1.25–1.57 ( <i>n</i> = 401)	9.7 (9.4, 10.0)	0.008	9.0 (8.7, 9.3)	—
1.58–1.86 ( <i>n</i> = 373)	9.4 (9.1, 9.7)	<0.001	8.9 (8.6, 9.2)	—
1.87–2.27 ( <i>n</i> = 412)	9.1 (8.8, 9.4)	<0.001	8.9 (8.6, 9.1)	—
$\geq 2.28$ ( <i>n</i> = 392)	9.1 (8.8, 9.3)	<0.001	9.2 (8.9, 9.5)	—
		<0.001 <sup>5</sup>		0.25 <sup>5</sup>
Alcohol intake ( $\text{g/d}$ )				
0 ( <i>n</i> = 484) <sup>4</sup>	9.4 (9.1, 9.7)		9.4 (9.2, 9.6)	
0.1–4.9 ( <i>n</i> = 571)	9.3 (9.1, 9.5)	0.60	9.3 (9.1, 9.5)	0.56
5.0–14.9 ( <i>n</i> = 458)	9.4 (9.1, 9.7)	0.98	9.4 (9.2, 9.6)	0.94
15.0–24.9 ( <i>n</i> = 152)	10.0 (9.5, 10.5)	0.03	9.9 (9.5, 10.3)	0.05
$\geq 25.0$ ( <i>n</i> = 295)	9.9 (9.6, 10.3)	0.02	9.8 (9.6, 10.2)	0.02
		0.006 <sup>5</sup>		0.004 <sup>5</sup>
Caffeine intake ( $\text{mg/d}$ )				
<88 ( <i>n</i> = 392) <sup>4</sup>	8.9 (8.6, 9.1)		9.0 (8.8, 9.3)	
89–182 ( <i>n</i> = 392)	9.5 (9.2, 9.8)	0.003	9.6 (9.3, 9.9)	0.002
183–355 ( <i>n</i> = 392)	9.8 (9.5, 10.1)	<0.001	9.6 (9.3, 9.9)	0.002
356–419 ( <i>n</i> = 392)	9.5 (9.3, 9.8)	<0.008	9.4 (9.2, 9.7)	0.03
$\geq 420$ ( <i>n</i> = 392)	9.9 (9.6, 10.2)	<0.001	9.8 (9.5, 10.0)	<0.001
		<0.001 <sup>5</sup>		<0.001 <sup>5</sup>

<sup>1</sup> Adjusted for age, sex, serum creatinine concentration, and folate, vitamin B-12, and vitamin B-6 intake (except for the alcohol and caffeine analyses, which were adjusted for plasma concentrations, not intake, of the B vitamins).

<sup>2</sup> Analyses are based on the subset of subjects who did not consume supplements containing B vitamins.

<sup>3</sup>  $\bar{x}$  (95% CI).

<sup>4</sup> Reference category for statistical comparisons.

<sup>5</sup> *P* for trend for linear regression coefficient of continuous variable.

<sup>6</sup> Also adjusted for use of vitamin B supplements.

<sup>7</sup> *F* statistic from analysis of variance was not significant (*P* > 0.05) and individual comparisons are not presented.

was not significantly associated with tHcy after inclusion of riboflavin intake in the regression model. This was a consequence of the strong correlation between vitamin B-6 and riboflavin intake (correlation coefficient = 0.81;  $P < 0.001$ ).

## DISCUSSION

Our results confirm the observed associations between fasting plasma tHcy concentration and its accepted determinants, including age, sex, serum creatinine, folate, and vitamin B-12. In the Framingham Offspring cohort, higher tHcy concentrations were associated with older age and male sex, which is consistent with observations from other large, population-based samples of men and women in the United States (3, 29), Norway (30), and the United Kingdom (14). Serum creatinine is consistently one of the strongest determinants of fasting tHcy concentrations (13–15). Also, as expected, folate and vitamin B-12 were associated with maintenance of low tHcy concentrations. Dietary vitamin B-12 was not associated with tHcy, which was also the case in the older Framingham Study cohort (3). Our results also provide support for other potential nutritional and nonnutritional determinants of fasting tHcy concentrations, including vitamin B-6, riboflavin, caffeine, and alcohol intakes; smoking status; and hypertension.

Several studies examined the relation between vitamin B-6 status and tHcy concentrations after a methionine load (1), but few studies did so under fasting conditions. Low vitamin B-6 status is believed to result in higher tHcy concentrations, particularly after a methionine load, because PLP is a coenzyme for 2 enzymes—cystathionine  $\beta$ -synthase and  $\gamma$ -cystathionase—that irreversibly convert homocysteine to cysteine (31). Vitamin B-6 may also affect tHcy concentrations through its role as a cofactor for serine hydroxymethyltransferase, an enzyme responsible for the transfer of a methyl group from serine to tetrahydrofolate (THF) to form methylene-THF. This latter compound, when reduced by the riboflavin-dependent enzyme methylenetetrahydrofolate reductase, is converted to 5-methyl-THF, which serves as the methyl donor for remethylation of homocysteine to methionine. In the Framingham Offspring cohort, PLP concentrations  $<40$  nmol/L, or intakes  $<1.6$  mg/d, were significantly related to higher fasting tHcy concentrations, but the relation between PLP and tHcy disappeared after adjustment for smoking status, and the relation between vitamin B-6 intake and tHcy disappeared after adjustment for riboflavin intake. Giraud et al (32) reported that plasma PLP concentration was significantly lower in cigarette smokers but that erythrocyte PLP and other B-6 vitamin concentrations appeared to be unaffected by smoking. One earlier study reported an association between PLP and fasting tHcy concentration that was attenuated, but still significant, after adjustment for several factors, including plasma folate and vitamin B-12 concentrations but not smoking status (33). Experimental studies relating vitamin B-6 supplementation or depletion with fasting tHcy concentrations had equivocal results (34, 35).

We observed a modest association between dietary riboflavin and tHcy. Although the importance of riboflavin in homocysteine metabolism is recognized (16), the relation between homocysteine and riboflavin received little attention in previous human studies (36–38). Riboflavin, in the form of flavin adenine dinucleotide, is a cofactor for methylenetetrahydrofolate reductase. As described above, this enzyme converts methylenetetrahydrofolate to methyl-THF, which is required for the remethylation of homocysteine to methionine.

Persons who regularly take vitamin B supplements have lower tHcy concentrations than do persons who do not (13, 30, 36–40). In the present study, use of vitamin B supplements was also associated with significantly lower tHcy concentrations.

Acute methionine loading increases circulating tHcy concentrations (1), but there is little evidence that short-term feeding of physiologic amounts of protein or methionine has any effect on tHcy concentrations (41, 42). We observed an inverse association between tHcy and protein and methionine consumption, but these associations largely disappeared after adjustment for dietary intakes of vitamin B-6 and folate. Stolzenberg-Solomon et al (17) also reported that total protein intake was inversely associated with tHcy concentrations after adjustment for dietary folate intake, but they did not adjust for vitamin B-6 intake. Shimakawa et al (37) did not see any association between protein or methionine intake and tHcy concentrations in participants in the Atherosclerosis Risk in Communities (ARIC) study.

There was a modest positive association between alcohol intake and fasting tHcy concentrations in this cohort, and significant positive associations were seen between tHcy and consumption of liquor and red wine, but not beer and white wine. This finding is similar to the results of a recent randomized trial that showed consumption of liquor and red wine, but not beer, raised tHcy concentrations (18).

Our observation that consumption of caffeine, coffee, and cola was positively associated with tHcy concentrations is supported by 2 earlier reports of a positive association between coffee consumption and tHcy concentrations (17, 19), although no association was seen between coffee consumption and tHcy in the ARIC cohort (36). A recent randomized trial showed that 1 L unfiltered coffee/d for 2 wk increased fasting tHcy concentrations by 10% (1.2  $\mu$ mol/L) (43). In contrast with our finding of a positive association with other caffeine-containing beverages, we saw a weak inverse association between tea consumption and tHcy. Tea is also considered to be an important source of caffeine, although the amount per serving is less than that in coffee. Our observation is consistent with data from the Hordaland Homocysteine Study (19). It is possible that the presence of modest amounts of folate in brewed tea (up to 20  $\mu$ g/150 mL) might offset any small effect of caffeine on homocysteine metabolism (44).

The relation between BMI and tHcy concentrations suggested that persons with the largest weight-for-height (BMI  $\geq 30.7$ ) had slightly greater plasma tHcy concentrations than did those with a BMI  $< 30.7$ . Koehler et al (40) also reported a weak positive relation between BMI and tHcy concentrations, but Lussier-Cacan et al (15) observed no association. The Hordaland Homocysteine Study investigators reported a U-shaped association between BMI and tHcy concentrations that disappeared after adjustment for other determinants of tHcy concentrations (30).

We also observed strong positive associations between tHcy concentrations and both cigarette smoking and use of antihypertensive medications, but no association with blood pressure. The association with cigarette smoking agrees with the association reported in the Hordaland Homocysteine Study (30). The relation with antihypertensive medication was not likely due to impaired renal function because the association was completely unaffected by adjustment for serum creatinine concentrations. Positive associations between tHcy and blood pressure were reported in the Hordaland Homocysteine Study (30) and by Brattstrom et al (13). The association in the latter study disappeared after multivariate adjustment for age, sex, and circulating concentrations of creati-


TABLE 4

Geometric mean total plasma homocysteine by intake of caffeine and alcohol-containing beverages

Beverage	Total plasma homocysteine			
	Age and sex adjusted	<i>P</i>	Multivariable adjusted <sup>1</sup>	<i>P</i>
	$\mu\text{mol/L}$		$\mu\text{mol/L}$	
Coffee (cups)				
<1/mo ( <i>n</i> = 359) <sup>2</sup>	9.0 (8.7, 9.3) <sup>3</sup>		9.1 (8.8, 9.4)	
1–3/mo ( <i>n</i> = 86)	9.3 (8.7, 9.9)	0.32	9.4 (8.9, 10.0)	0.26
1–6/wk ( <i>n</i> = 197)	9.2 (8.9, 9.7)	0.27	9.4 (9.0, 9.7)	0.26
1/d ( <i>n</i> = 296)	9.5 (9.2, 9.9)	0.02	9.6 (9.3, 9.9)	0.03
2–3/d ( <i>n</i> = 736)	9.6 (9.4, 9.8)	<0.001	9.5 (9.3, 9.7)	0.02
≥4/d ( <i>n</i> = 273)	10.0 (9.7, 10.4)	<0.001	9.9 (9.5, 10.2)	<0.001
		<0.001 <sup>4</sup>		<0.001 <sup>4</sup>
Decaffeinated coffee (cups)				
<1/mo ( <i>n</i> = 1100) <sup>2</sup>	9.7 (9.6, 9.9)		9.6 (9.5, 9.8)	
1–3/mo ( <i>n</i> = 203)	9.1 (8.8, 9.5)	0.009	9.3 (9.0, 9.7)	0.13
1–6/wk ( <i>n</i> = 274)	9.2 (8.9, 9.6)	0.02	9.4 (9.1, 9.7)	0.16
1/d ( <i>n</i> = 167)	8.8 (8.4, 9.2)	<0.001	9.0 (8.6, 9.4)	0.003
≥2/d ( <i>n</i> = 191)	9.5 (9.1, 10.0)	0.44	9.5 (9.1, 9.9)	0.51
		0.40 <sup>4</sup>		0.43 <sup>4</sup>
Cola with caffeine (360-mL cans)				
<1/mo ( <i>n</i> = 756) <sup>2</sup>	9.4 (9.1, 9.6)		9.4 (9.2, 9.6)	
1–3/mo ( <i>n</i> = 312)	9.5 (9.1, 9.8)	0.58	9.6 (9.3, 9.9)	0.33
1–6/wk ( <i>n</i> = 578)	9.5 (9.2, 9.7)	0.46	9.4 (9.2, 9.7)	0.69
1/d ( <i>n</i> = 144)	9.6 (9.2, 10.2)	0.31	9.4 (9.1, 9.9)	0.84
≥2/d ( <i>n</i> = 130)	10.5 (10.0, 11.1)	<0.001	10.3 (9.8, 10.8)	<0.001
		<0.001 <sup>4</sup>		<0.001 <sup>4</sup>
Tea (cups)				
<1/mo ( <i>n</i> = 931) <sup>2</sup>	9.7 (9.5, 9.9)		9.7 (9.5, 9.8)	
1–3/mo ( <i>n</i> = 277)	9.3 (8.9, 9.6)	0.02	9.4 (9.1, 9.7)	0.11
1–6/wk ( <i>n</i> = 359)	9.2 (8.9, 9.5)	0.006	9.3 (9.0, 9.5)	0.02
1/d ( <i>n</i> = 194)	9.5 (9.1, 9.9)	0.30	9.4 (9.0, 9.8)	0.24
≥2/d ( <i>n</i> = 165)	9.2 (8.8, 9.7)	0.04	9.1 (8.8, 9.5)	0.02
		0.07 <sup>4</sup>		0.03 <sup>4</sup>
Red wine (120-mL glasses)				
<1/mo ( <i>n</i> = 1252) <sup>2</sup>	9.5 (9.3, 9.6)		9.5 (9.3, 9.6)	
1–3/mo ( <i>n</i> = 333)	9.3 (9.0, 9.6)	0.40	9.4 (9.1, 9.6)	0.55
1–6/wk ( <i>n</i> = 295)	9.7 (9.3, 10.0)	0.29	9.7 (9.4, 10.0)	0.22
≥1/d ( <i>n</i> = 62)	9.9 (9.2, 10.8)	0.25	9.8 (9.1, 10.5)	0.39
		0.03 <sup>4</sup>		0.05 <sup>4</sup>
White wine (120-mL glasses)				
<1/mo ( <i>n</i> = 1003) <sup>2</sup>	9.6 (9.4, 9.8)	— <sup>5</sup>	9.5 (9.4, 9.7)	—
1–3/mo ( <i>n</i> = 435)	9.3 (9.0, 9.6)	—	9.3 (9.1, 9.6)	—
1–6/wk ( <i>n</i> = 428)	9.5 (9.2, 9.7)	—	9.5 (9.3, 9.8)	—
≥1/d ( <i>n</i> = 80)	10.0 (9.3, 10.7)	—	9.8 (9.2, 10.4)	—
		0.36 <sup>4</sup>		0.53 <sup>4</sup>
Beer (360-mL cans)				
<1/mo ( <i>n</i> = 1109) <sup>2</sup>	9.5 (9.3, 9.7)	—	9.4 (9.3, 9.6)	—
1–3/mo ( <i>n</i> = 263)	9.4 (9.1, 9.8)	—	9.6 (9.3, 9.9)	—
1–6/wk ( <i>n</i> = 396)	9.6 (9.3, 9.9)	—	9.5 (9.2, 9.8)	—
≥1/d ( <i>n</i> = 183)	9.5 (9.1, 10.0)	—	9.5 (9.1, 9.9)	—
		0.62 <sup>4</sup>		0.72 <sup>4</sup>
Liquor (shots)				
<1/mo ( <i>n</i> = 1117) <sup>2</sup>	9.4 (9.2, 9.6)		9.4 (9.2, 9.5)	
1–3/mo ( <i>n</i> = 299)	9.4 (9.1, 9.8)	0.85	9.4 (9.1, 9.7)	0.71
1–6/wk ( <i>n</i> = 379)	9.6 (9.3, 9.9)	0.34	9.5 (9.2, 9.8)	0.43
≥1/d ( <i>n</i> = 164)	10.2 (9.7, 10.7)	0.002	10.2 (9.8, 10.7)	<0.001
		<0.001 <sup>4</sup>		<0.001 <sup>4</sup>

<sup>1</sup> Adjusted for age, sex, serum creatinine concentration, and plasma folate, vitamin B-12, and pyridoxal phosphate concentrations.<sup>2</sup> Reference category for statistical comparisons.<sup>3</sup>  $\bar{x}$  (95% CI).<sup>4</sup> *P* for trend for linear regression coefficient of continuous beverage variable (servings/wk).<sup>5</sup> *F* statistic from analysis of variance was not significant (*P* > 0.05) and individual comparisons are not presented.

nine, folate, and vitamin B-12. Subjects in that study who were treated for hypertension also had significantly higher tHcy concentrations than did those who were not (13). Lussier-Cacan et al (15) also noted no association between blood pressure and tHcy.

Given the growing evidence that homocysteine is an independent risk factor for vascular disease (1, 2), we need to better understand the modifiable determinants of tHcy concentration. Until recently, population studies suggested that low folate status was the most important determinant of mild-to-moderate hyperhomocysteinemia (3, 4). Consequently, much of the research on determinants of tHcy concentrations focused on folate. However, the recent folic acid fortification of enriched grain products in the United States dramatically altered the prevalence of elevated tHcy concentrations associated with low folate (45). Because other determinants will now assume greater importance, we need to continue our efforts to identify additional risk factors for mild-to-moderate hyperhomocysteinemia. 

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