Demographic, health, lifestyle, and blood vitamin determinants of serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey, 1988–1994[1,2]

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ABSTRACT

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

Objective: Associations between serum tHcy and demographics, health and lifestyle factors, and blood vitamin concentrations were investigated.

Design: Data from the third National Health and Nutrition Examination Survey, 1988–1994 were used to examine associations in men ($n = 2965$) and women ($n = 3580$) between tHcy and age, sex, race-ethnicity, body mass index, systolic and diastolic blood pressures, alcohol consumption, supplement use, red blood cell (RBC) folate, and serum creatinine, folate, vitamin B-12, and cotinine (a measure of cigarette smoking).

Results: The unadjusted mean tHcy was 21.5% (≈1.9 μmol/L) higher in men than in women, 11.8% (≈1.1 μmol/L) higher in non-Hispanic whites than in Mexican Americans, 42% (≈3.7 μmol/L) higher in persons aged ≥ 70 y than in persons aged < 30 y, and 10.9% (≈1.0 μmol/L) higher in supplement nonusers than in supplement users. The tHcy concentration was negatively associated with serum folate ($P < 0.0001$ for trend), RBC folate ($P < 0.0001$ for trend), and serum vitamin B-12 ($P < 0.0036$ for trend) and was positively associated with alcohol consumption ($P < 0.0001$ for trend), serum cotinine ($P < 0.0001$ for trend), and systolic blood pressure ($P < 0.0001$ for trend). Consumption of hard liquor (but not of beer or wine) was positively associated with tHcy concentration ($P < 0.0001$ for trend).

Conclusions: In this population-based study, the significant predictors of tHcy concentration were sex, age, race-ethnicity, serum creatinine, systolic blood pressure, body mass index, hard-liquor consumption, smoking, supplement use, serum folate, RBC folate, and serum vitamin B-12. *Am J Clin Nutr* 2003;77:826–33.

KEY WORDS Homocysteine, third National Health and Nutrition Examination Survey, NHANES III, folate, red blood cell folate, vitamin B-12, creatinine, smoking, alcohol consumption, cotinine, age, race, ethnicity, supplements, blood pressure, body mass index, heart disease, coronary artery disease, cardiovascular disease, vascular disease

INTRODUCTION

Homocysteine is a nonessential sulfur-containing amino acid formed from the demethylation of an essential amino acid, methionine (1). Epidemiologic studies have shown that elevated total homocysteine (tHcy) concentration in the blood is an independent risk factor for occlusive vascular diseases (2–5). Several possible mechanisms that may underlie the positive association between homocysteine concentration and risk for heart disease include oxidation of LDL cholesterol, toxic effects on endothelial cells, impaired platelet activity, and increased smooth cell proliferation (2, 4, 6–8).

Coenzymes of folate, vitamin B-12, riboflavin, and pyridoxine vitamers are essential in homocysteine metabolism. Homocysteine is either remethylated to methionine or transsulfurated to cysteine. Folate, riboflavin, and vitamin B-12 coenzymes are needed for the remethylation pathway, whereas pyridoxine coenzyme is required for the transsulfuration pathway (9–12). Decreased circulating concentrations of folate, riboflavin, vitamin B-12, and pyridoxine are associated with increased serum tHcy concentration (10, 13). The association between red blood cell (RBC) folate and tHcy has received little attention. Serum creatinine, a measure of renal function, is positively associated with serum tHcy (14). An inverse association between the glomerular filtration rate and tHcy has also been reported (15).

Smoking is associated with elevated plasma tHcy concentrations (10). The possible association between tHcy and serum cotinine, a measure of cigarette smoking, has never been studied. Published reports on the relation between alcohol consumption and tHcy concentration are inconsistent (16). The potential association between body mass index (BMI; in kg/m^2) and tHcy has received little attention. So far, little has been published on the determinants of tHcy from population-based studies in the United States. We examined the data from the third National Health and Nutrition Examination Survey, 1988–1994 (NHANES III) for associations between serum tHcy concentrations and demographics, lifestyle and health factors, and blood vitamin concentrations.

SUBJECTS AND METHODS

Study sample

The NHANES III was a stratified probability survey of the noninstitutionalized US population conducted from 1988 to 1994. The

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data used for the current report were obtained from databases released for public use by the National Technical Information Service, Springfield, VA (17–20). A detailed description of the survey methodology was obtained from another database (21). The survey sample included 39 695 subjects. Of these, 86% were interviewed in their homes and 78% were also examined in the mobile examination clinics. Only subjects aged ≥ 17 y (n = 20 050) were included in the study because alcohol intake data were not collected for individuals < 17 y. Of these 20 050 individuals, those who answered yes to the diabetes question (n = 1614) were excluded. Diabetics were excluded because in the NHANES III population, diabetics had significantly higher serum tHcy concentrations than did nondiabetics (data not shown). Pregnant (n = 338) and lactating (n = 100) women were excluded from the analysis because, during pregnancy, tHcy decreases by ≈50% (22) and returns to normal concentrations within 2–4 d postpartum (23). Therefore, because these groups of potential subjects were excluded, the results in this study represent the apparently healthy population. We also excluded 11 480 participants because they had missing data for tHcy or the covariates that were examined. Thus, the current study included a total of 6545 subjects (2965 men and 3580 women).

Measurements

Depending on the age of the participant, data were collected on demographics, physical function, health condition, lifestyle behaviors, biochemical measurements of blood and urine, body measurements, and dietary intake. Blood was collected by venipuncture in the mobile examination clinics. Of 6545 participants, ≈62% (n = 4067) had fasted for ≥ 8 h, 29% (n = 1896) had fasted for 5 h to < 8 h, and ≈9% (n = 575) had fasted for < 5 h. Data on duration of fasting were missing for 7 participants. Duration of fasting had no measurable effect on serum tHcy concentration (24). Serum was separated by centrifugation (115 × g for 15 min at room temperature) after the blood samples had been held at room temperature for 30–60 min. Serum samples were frozen at −20 °C and transported on dry ice to the Centers for Disease Control and Prevention for priority analyses. After completion of priority analyses, surplus serum samples were stored at −70 °C for 8 mo to 3 y. The tHcy concentrations in these surplus serum samples were measured in phase 2 of the NHANES III (1991–1994) at the US Department of Agriculture Human Nutrition Research Center on Aging after approval by the New England Medical Center Human Investigations Review Committee. Serum tHcy concentration was measured with the HPLC method (25). Serum folate, RBC folate, and vitamin B-12 concentrations were measured by using radioassays (26). The vitamin B-12 concentration was measured in phase 2 of the NHANES III.

Blood pressure was measured with a mercury sphygmomanometer (WA Baum Co Inc, Copiague, NY) according to the standardized protocol recommended by the American Heart Association (27). Participants were asked to report their consumption of beer ("lite" beer included), wine (wine coolers, sangria, and champagne included), and hard liquor (gin, rum, whiskey, tequila, vodka, liqueurs, etc) in times/mo. One drink of alcohol was described as 12 oz (360 mL) beer, 4 oz (120 mL) wine, or 1 oz (30 mL) hard liquor. The total number of alcoholic drinks consumed by participants was computed by adding the numbers of drinks of beer, wine, and hard liquor. Participants who reported ≥ 1 total alcoholic drink/mo were categorized as alcohol drinkers, whereas participants who reported none were categorized as alcohol nondrinkers. Alcohol drinkers were further classified as light drinkers, moderate drinkers, or heavy drinkers if their reported total consumption of alcohol was 1–30 drinks/mo, 31–60 drinks/mo, or > 60 drinks/mo, respectively.

We used serum cotinine concentration as a measure of the intensity of smoking; a previous study showed that serum cotinine was correlated with the number of cigarettes smoked (28). Serum cotinine was measured with an enzyme-linked immunoassay. A serum cotinine concentration ≥ 14 μg/L was used as an indicator of active smoking (29). Participants who answered "yes" to the question "have you taken vitamins/minerals in past month?" were regarded as supplement users. In the NHANES III, a total of 7381 individuals were reported to be taking vitamin/mineral supplements. We tested sex, age, race-ethnicity, BMI, systolic and diastolic blood pressures, alcohol consumption, smoking, vitamin/mineral supplement use, and concentrations of serum creatinine, serum folate, RBC folate, and serum vitamin B-12 as potential determinants of serum tHcy concentration.

Statistical analyses

The statistical analyses were performed with SAS for WINDOWS, version 8.0 (SAS Institute Inc, Cary, NC). The serum tHcy data are presented as means with 95% CIs. The CIs were calculated by using Scheffe's procedure; we used Scheffe's procedure because it gives the most conservative CI. BMI, alcohol consumption, serum cotinine, serum creatinine, systolic and diastolic blood pressure, serum folate, RBC folate, and serum vitamin B-12 were analyzed as continuous variables. Age, sex, race-ethnicity, and vitamin/mineral supplement use were analyzed as discrete variables. In the NHANES III, individuals aged > 90 y were categorized as 90 y to protect the confidentiality of survey participants. This precluded us from using age as a continuous variable. In our study sample, 38 individuals were classified as 90 y. We categorized the study sample into 4 age groups: < 30 y, 30 to < 50 y, 50 to < 70 y, and ≥ 70 y.

We tested for significant differences between men and women on selected characteristics by using two-tailed t tests. Multivariate linear regression was used to evaluate the simultaneous effects of selected predictors of serum tHcy on serum tHcy. The serum tHcy concentration was determined according to the levels of various predictors of serum tHcy. All continuous predictor variables except alcohol consumption were divided into quartiles. Multivariate analysis of variance was used to determine the sex-, age-, and race-ethnicity-adjusted and multivariate adjusted means for serum tHcy concentration within the levels of various predictors of serum tHcy. Pairwise comparisons were performed with Tukey's studentized test within the levels of various predictors of serum tHcy. Tukey's test was used because this test controls for type I experiment-wise error.

Statistical significance for linear trend and regression coefficients (β) were determined for continuous predictor variables with multiple linear regression analysis. Further, separate linear regression was performed by replacing total alcoholic drinks/mo with number of beer, wine, and hard-liquor drinks/mo as continuous predictor variables in the model. Pearson's product-moment correlation coefficients (r) were calculated to evaluate the associations between continuous variables and serum tHcy concentration. Statistical significance was set at $P < 0.05$.

RESULTS

Selected subject characteristics are shown in **Table 1**. The study sample was 45% male and 55% female. The mean ages of men and women were not significantly different. On the basis of serum cotinine concentrations (≥ 14.0 μg/L), ≈16% of men and ≈13%

TABLE 1
Selected characteristics of the study population[1]

Characteristic	Men (n = 2965)	Women (n = 3580)
Race-ethnicity		
Non-Hispanic white (n)	1017	1414
Non-Hispanic black (n)	894	1136
Mexican American (n)	929	831
Other (n)	125	199
Alcohol consumption		
Yes (n)[2]	1777	1349
No (n)	1188	2231
Smoking		
Yes (n)[3]	1024	816
No (n)[4]	1941	2764
Supplement use		
Yes (n)	967	1495
No (n)	1998	2085
Age (y)	44.4 (43.7, 45.1)[5]	44.7 (44.1, 45.4)
Weight (kg)	80.3 (79.7, 80.9)	71.0 (70.4, 71.6)[6]
Height (cm)	173.5 (173.2, 173.8)	160.6 (160.4, 160.9)[6]
BMI (kg/m^2)	26.2 (26.4, 26.8)	27.5 (27.3, 27.7)[6]
Systolic BP (mm Hg)	126.5 (125.9, 127.1)	121.9 (121.3, 122.6)[7]
Diastolic BP (mm Hg)	76.8 (76.4, 77.2)	72.4 (72.1, 72.8)[6]
Serum creatinine (μmol/L)	106.2 (105.3, 107.2)	86.0 (85.5, 86.5)[6]
Alcohol intake		
Total alcohol intake (drinks/mo)	11.4 (10.6, 12.3)	4.1 (3.7, 4.5)[6]
Beer (drinks/mo)	7.7 (7.0, 8.3)	2.1 (1.7, 2.4)[6]
Wine (drinks/mo)	1.1 (0.9, 1.2)	1.2 (1.1, 1.4)
Hard liquor (drinks/mo)	2.7 (2.2, 3.2)	0.8 (0.7, 0.9)[6]
Serum cotinine (μg/L)	79.9 (74.6, 85.2)	53.0 (48.8, 57.2)[6]
Serum folate (nmol/L)	13.8 (13.4, 14.2)	16.1 (15.7, 16.6)[6]
RBC folate (nmol/L)	399.1 (391.7, 406.4)	428.6 (420.5, 436.8)[6]
Serum vitamin B-12 (pmol/L)	373.3 (364.0, 382.7)	463.6 (395.2, 531.9)[6]

[1] BP, blood pressure; RBC, red blood cell.
[2] Defined as consumption of ≥1 drink/mo.
[3] Subjects with a serum cotinine concentration ≥14 μg/L.
[4] Subjects with a serum cotinine concentration <14 μg/L.
[5] $\bar{x}$; 95% CI in parentheses.
[6,7] Significantly different from men (two-tailed t test): [6] $P < 0.0001$, [7] $P = 0.0104$.

of women were categorized as smokers and ≈27% of men and ≈21% of women were reported as alcohol nondrinkers. The total number of alcoholic drinks/mo was significantly higher for men than for women. Men consumed significantly more beer and hard-liquor drinks/mo than did women. However, wine consumption did not differ significantly between men and women. About 33% of study participants reported use of vitamin/mineral supplements. BMI was significantly higher in women than in men. Systolic and diastolic blood pressures, serum creatinine, and serum cotinine concentrations were significantly higher in men than in women. Serum folate, RBC folate, and serum vitamin B-12 concentrations were significantly higher in women than in men.

Serum tHcy concentrations of the study participants by sex, age, and race-ethnicity are shown in **Table 2**. For all subjects, serum tHcy concentrations ranged from 3.0 to 132 μmol/L. The mean serum tHcy was 21.1% higher in men than in women, and this difference was significant ($P < 0.0001$). However, after adjustment for multivariates (BMI, systolic and diastolic blood pressures, serum creatinine, total alcohol intake, vitamin/mineral supplement use, serum cotinine, serum folate, RBC folate, and serum vitamin B-12), sex was not significantly associated with serum tHcy ($P = 0.1713$). Age was significantly associated with serum tHcy ($P < 0.0001$). Mean serum tHcy was 40.4% (≈3.6 μmol/L) higher in individuals aged ≥70 y, 18.0% (≈1.6 μmol/L) higher in individuals aged 50 to <70 y, and 2.2% (≈0.2 μmol/L) higher in individuals aged 30 to <50 y than it was in subjects aged <30 y. Among race-ethnicity groups, non-Hispanic blacks had the lowest serum tHcy. Non-Hispanic whites had 11.2% higher and 3.7% higher serum tHcy than non-Hispanic blacks and Mexican-Americans, respectively.

Associations between serum tHcy and health and lifestyle factors are shown in **Table 3**. Among all the variables examined, serum creatinine had the strongest linear association with tHcy ($P < 0.0001$ for linear trend, $\beta = 0.0631$, $r = 0.302$). The difference in serum tHcy between the lowest and highest quartiles of serum creatinine was ≈4.1 μmol/L (53.2%). Systolic blood pressure showed a positive association with serum tHcy ($P < 0.0001$ for linear trend, $\beta = 0.0568$, $r = 0.2$). Diastolic blood pressure showed no significant association with serum tHcy when other variables were considered in the multivariate analysis ($P = 0.7969$). Multivariate adjusted serum tHcy showed a negative association with BMI ($P < 0.0054$).

Alcohol consumption was positively associated with serum tHcy ($P < 0.0001$ for linear trend, $\beta = 0.0178$, $r = 0.105$). Subjects who consumed an average of >60 drinks/mo had significantly higher serum tHcy concentrations than did alcohol nondrinkers ($P < 0.0001$). Moderate drinkers (31–60 drinks/mo) had significantly higher serum tHcy than did alcohol nondrinkers ($P < 0.0001$). Hard-liquor consumption was positively associated with serum tHcy concentration ($P < 0.0001$ for linear trend, $\beta = 0.0681$, $r = 0.172$). However, serum tHcy was not significantly associated with beer consumption ($P = 0.1984$ for linear trend, $r = 0.02$) or wine consumption ($P = 0.8770$ for linear trend, $r = 0.008$).

Subjects who did not use supplements had 10.9% higher serum tHcy than did those who used supplements, but when other variables were included in the multivariate model, supplement use was not significantly associated with serum tHcy ($P = 0.4874$).

Serum cotinine was positively associated with tHcy concentration ($P < 0.0001$ for linear trend, $\beta = 0.0027$, $r = 0.112$). Subjects in the highest quartile of serum cotinine concentrations had significantly higher serum tHcy than did subjects in the lowest serum cotinine quartile ($P < 0.05$). There were no significant differences in serum tHcy among the first, second, and third serum cotinine quartiles.

Associations between serum tHcy and blood concentrations of vitamins are shown in **Table 4**. Serum folate showed a negative linear association with serum tHcy ($P < 0.0001$ for linear trend, $\beta = -0.0441$, $r = -0.125$). The multivariate-adjusted mean for serum tHcy was 34.8% (≈3.2 nmol/L) higher in the lowest quartile of serum folate than in the highest quartile of serum folate. A similar negative association was found between serum tHcy and RBC folate ($P < 0.0001$ for linear trend, $\beta = -0.0024$, $r = -0.127$). The association between serum tHcy and serum vitamin B-12 was also negative, but was not as strong ($P < 0.0036$ for linear trend, $\beta = -0.0001$, $r = -0.044$).

DISCUSSION

Our findings from the NHANES III data support previously reported associations between serum tHcy and sex, age, serum folate, serum vitamin B-12, alcohol consumption, smoking, serum creatinine, and supplement use (3, 10, 30–32). Among all the con-

TABLE 2
Mean serum total homocysteine (tHcy) concentration by demographic factors in the third National Health and Nutrition Examination Survey

	Unadjusted analysis[1]		Multivariate-adjusted analysis[2]	
	Serum tHcy	P[3]	Serum tHcy	P[4]
	μmol/L		*μmol/L*	
Sex				
Male (n = 2965)[5]	10.9 (10.6, 11.1)[6]		10.5 (10.2, 10.9)	
Female (n = 3580)	9.0 (8.8, 9.2)[7]		10.3 (10.0, 10.7)	
		<0.0001		0.1713
Age (y)				
<30 (n = 1789)[5]	8.9 (8.5, 9.3)		9.1 (8.7, 9.4)	
30 to <50 (n = 2360)	9.0 (8.7, 9.4)		9.4 (9.1, 9.8)	
50 to <70 (n = 1452)	10.6 (10.1, 11.0)[7]		10.8 (10.4, 11.2)[7]	
≥70 (n = 944)	12.5 (12.0, 13.1)[7]		12.5 (12.0, 13.0)[7]	
		<0.0001		<0.0001
Race-ethnicity				
Non-Hispanic white (n = 2431)[5]	10.4 (10.1, 10.7)		10.6 (10.3, 10.9)	
Non-Hispanic black (n = 2030)	9.6 (9.3, 10.0)[7]		10.0 (9.6, 10.3)[7]	
Mexican American (n = 1760)	9.3 (8.9, 9.7)[7]		10.4 (10.1, 10.8)	
Others (n = 324)	9.6 (8.7, 10.5)[7]		10.7 (10.2, 11.3)	
		<0.007		<0.0001

[1] ANOVA with age, sex, and race-ethnicity as independent variables and tHcy as the dependent variable.
[2] ANOVA for sex, age, and race-ethnicity adjusted for BMI, systolic and diastolic blood pressures, serum creatinine, alcohol consumption, serum cotinine, supplement use, serum folate, red blood cell folate, and vitamin B-12.
[3] Effect of age, sex, or race-ethnicity on tHcy (ANOVA).
[4] Effect of age, sex, or race-ethnicity adjusted for BMI, systolic and diastolic blood pressures, serum creatinine, alcohol consumption, serum cotinine, supplement use, serum folate, red blood cell folate, and vitamin B-12 covariates on tHcy (multivariate ANOVA).
[5] Used as a reference group for comparison within the categories of the variable.
[6] $\bar{x}$; 95% CI in parentheses.
[7] Significantly different from the reference group within the variable, $P < 0.05$ (Tukey's studentized pairwise comparison test).

tinuous variables we examined, serum creatinine showed the strongest association with serum tHcy, and serum vitamin B-12 showed the least association with serum tHcy. The inverse associations we found between tHcy and both serum folate and vitamin B-12 confirm previously reported findings (3, 10, 30–32). The inverse association between tHcy and RBC folate was rather weak, suggesting that RBC folate is not a strong predictor of tHcy. Supplement users had serum tHcy concentrations that were ≈1.0 μmol/L lower than the concentrations of supplement nonusers. However, the association between supplement use and tHcy disappeared when other variables were included in the multivariate analysis.

Although the mean serum tHcy concentration of men was significantly higher (by ≈1.9 μmol/L) than that of women, the association between sex and serum tHcy disappeared when other variables were included in the multivariate analysis. In this study, men had a significantly higher mean serum creatinine (by ≈20.2 μmol/L) than did women. Compared with women, men had lower mean concentrations of serum folate (by ≈2.3 nmol/L), RBC folate (by ≈29.5 nmol/L), and serum vitamin B-12 (by ≈90.3 pmol/L). Men consumed more alcohol (by ≈7.3 drinks/mo) and had a higher mean serum cotinine concentration (by ≈26.9 μg/L) than did women. Thus, the difference in serum tHcy between men and women can be explained by the differences in alcohol consumption, RBC folate concentration, and serum concentrations of folate, vitamin B-12, creatinine, and cotinine.

In this study, subject age was positively associated with serum tHcy concentration. For example, serum tHcy was ≈1.5 μmol/L (16.5%) higher in subjects aged 50–70 y than in those aged 30–50 y and was ≈2.0 μmol/L (18.9%) higher in subjects aged ≥70 y than in those aged 50–70 y. These results support the previously reported association between tHcy and age. In the Hordaland study, plasma tHcy in 40–42-y-olds was 10.8 μmol/L in men and 9.1 μmol/L in women. In 65–67-y-olds, tHcy was 12.3 μmol/L in men and 11.0 μmol/L in women (3). In the Framingham Study, tHcy was 23% higher in subjects aged ≥65 y than in subjects aged <45 y (10). Elevated tHcy in older populations may result from reduced activity of cystathionine β-synthase (EC 4.2.1.22) (33); this vitamin-B-6-dependent enzyme is essential for transsulfuration of homocysteine to cystathionine. Also, an age-dependant increase in tHcy can be related to a decline in renal function (34). Furthermore, elevated serum tHcy in older populations can also be attributed to low blood folate concentrations (35) and an increased incidence of vitamin B-12 deficiency resulting from malabsorption of vitamin B-12 by the aging gut (36).

In this study, race-ethnicity was associated with serum tHcy. Among all the race-ethnicity groups examined, non-Hispanic whites had the highest serum tHcy concentrations. Mean serum tHcy concentrations in non-Hispanic whites were ≈0.8 and ≈1.1 μmol/L higher than those of non-Hispanic blacks and Mexican-Americans, respectively. Elevated tHcy can be attributed to point mutation (cytosine to thymidine substitution at nucleotide 677) in the gene that encodes N^5,N^{10}-methylenetetrahydrofolate reductase (MTHFR; EC 1.7.99.5) (37). The 677C→T mutation results in reduced activity of MTHFR; activity is reduced to ≈34% in *TT* and ≈71% in *CT* relative to *CC* (38). MTHFR is required for the conversion of N^5,N^{10}-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is a methyl donor for remethylation of homocysteine to methionine. Individuals who are homozygous for the 677C→T mutation have increased circulat-

TABLE 3
Mean serum total homocysteine (tHcy) concentrations by health and lifestyle factors in the third National Health and Nutrition Examination Survey[1]

	Analysis adjusted for age, sex, and race-ethnicity[2]		Multivariate-adjusted analysis[3]	
	Serum tHcy	P[4]	Serum tHcy	P[5]
	μmol/L		*μmol/L*	
BMI (kg/m^2)[6]				
<22.9 (n = 1600)[7]	9.7 (9.3, 10.1)[8]		10.8 (10.4, 11.1)	
22.9 to <26.2 (n = 1635)	10.0 (9.6, 10.4)		10.5 (10.2, 10.9)	
26.2 to <30.1 (n = 1648)	10.0 (9.6, 10.4)		10.3 (9.9, 10.7)[9]	
≥30.1 (n = 1662)	9.6 (9.3, 10.0)		10.2 (9.5, 10.6)[9]	
		<0.6123		0.0054
Systolic BP (mm Hg)[6]				
<110 (n = 1501)[7]	8.5 (8.1, 9.0)		10.3 (9.8, 10.7)	
110 to <120 (n = 1647)	9.3 (8.9, 9.7)[9]		10.3 (9.9, 10.7)	
120 to <134 (n = 1721)	9.8 (9.4, 10.2)[9]		10.2 (9.9, 10.6)	
≥134 (n = 1676)	11.6 (11.2, 11.9)[9]		11.0 (10.6, 11.4)[9]	
		<0.0001		0.0002
Diastolic BP (mm Hg)[6]				
<67 (n = 1467)[7]	9.2 (8.8, 9.6)		10.4 (10.0, 10.8)	
67 to <74 (n = 1693)	9.7 (9.3, 10.0)[9]		10.5 (10.1, 10.8)	
74 to <81 (n = 1642)	9.8 (9.4, 10.2)[9]		10.4 (10.0, 10.8)	
≥81 (n = 1743)	10.6 (10.2, 11.0)[9]		10.5 (10.1, 10.9)	
		<0.0210		0.7969
Serum creatinine (μmol/L)[6]				
<79.6 (n = 749)[7]	7.7 (7.1, 8.2)		9.1 (8.6, 9.5)	
79.6 to <88.4 (n = 1203)	8.4 (7.9, .8.8)[9]		9.7 (9.3, 10.1)[9]	
88.4 to <106.1 (n = 2612)	9.6 (9.3, 9.9)[9]		10.6 (10.3, 11.0)[9]	
≥106.1 (n = 1981)	11.8 (11.5, 12.2)[9]		12.4 (12.0, 12.8)[9]	
		<0.0001		<0.0001
Alcohol intake (drinks/mo)				
0 (n = 3419)[7]	9.8 (9.5, 10.0)		9.9 (9.7, 10.1)	
1–30 (n = 2726)	9.7 (9.4, 10.0)		9.9 (9.7, 10.2)	
31–60 (n = 277)	10.9 (10.0,11.9)[9]		10.4 (9.8, 11.0)	
>60 (n = 123)	10.8 (10.4,13.3)[9]		11.5 (10.7, 12.4)[9]	
		<0.017		0.0009
Vitamin/mineral supplement use[10]				
Yes (n = 2462)[7]	9.2 (9.0, 9.5)		10.4 (10.0, 10.7)	
No (n = 4083)	10.2 (10.0, 10.4)[9]		10.5 (10.2, 10.8)	
		<0.0001		0.4874
Serum cotinine (ng/mL)[6]				
<0.087 (n = 1636)[7]	9.6 (9.2, 10.0)		10.2 (9.9, 10.6)	
0.087 to <0.331 (n = 1622)	9.5 (9.1, 9.9)		10.2 (9.5, 10.6)	
0.331 to <47.1 (n = 1648)	9.4 (9.0, 9.8)		10.3 (9.9, 10.7)	
≥47.1 (n = 1642)	10.8 (10.4, 11.2)[9]		11.0 (10.6, 11.4)[9]	
		<0.0001		0.0001

[1] BP, blood pressure.
[2] Analysis of covariance with age, sex, and race-ethnicity as independent variables and tHcy as the dependent variable.
[3] ANOVA adjusted for age, sex, race-ethnicity, BMI, systolic and diastolic BPs, serum creatinine, alcohol intake, serum cotinine, supplement use, serum folate, red blood cell folate, and vitamin B-12.
[4] Effect of the age-, sex-, and race-ethnicity–adjusted variable on tHcy (analysis of covariance).
[5] Effect of the multivariate-adjusted variable on tHcy (multivariate ANOVA).
[6] Categorized by quartile.
[7] Used as a reference group for comparison within the categories of the variable.
[8] $\bar{x}$; 95% CI in parentheses.
[9] Significantly different from the reference group within the variable, $P < 0.05$ (Tukey's studentized pairwise comparison test).
[10] Discrete variable.

ing tHcy when dietary folate intake is low (39). The point mutations in the *MTHFR* gene are more common in whites than in blacks (11). This may partially, if not solely, explain the elevated concentrations of serum tHcy in non-Hispanic whites compared with those in other ethnic groups.

Several studies have reported a positive association between cigarette smoking and tHcy concentration (3, 10, 30, 31). To our knowledge, this study was the first to show a positive association between serum cotinine concentration and serum tHcy. Cotinine is a metabolite of nicotine with a half-life of ≈20 h (40). Absorbed

TABLE 4
Mean serum total homocysteine (tHcy) concentrations by blood concentrations of vitamins in the third National Health and Nutrition Examination Survey[1]

	Analysis adjusted for age, sex, and race-ethnicity[2]		Multivariate-adjusted analysis[3]	
	Serum tHcy	P[4]	Serum tHcy	P[5]
	μmol/L		*μmol/L*	
Serum folate (nmol/L)[6]				
<7.7 (n = 1520)	12.0 (11.6, 12.3)[7,8]		12.4 (12.0, 12.8)[8]	
7.7 to <11.3 (n = 1747)	9.7 (9.3, 10.1)[8]		10.3 (10.0, 10.7)[8]	
11.3 to <17.7 (n = 1633)	9.1 (8.8, 9.5)[8]		9.8 (9.4, 10.1)[8]	
≥17.7 (n = 1645)[9]	8.7 (8.3, 9.1)		9.2 (8.8, 9.6)	
		<0.0001		<0.0001
RBC folate (nmol/L)[6]				
<267.4 (n = 1624)	11.2 (10.8, 11.6)[8]		11.0 (10.6, 11.4)[8]	
267.4 to <358.0 (n = 1631)	9.9 (9.6, 10.3)[8]		10.6 (10.2, 10.9)[8]	
358.0 to <496.3 (n = 1640)	9.4 (9.0, 9.8)[8]		10.4 (10.0, 10.7)[8]	
≥496.3 (n = 1650)[9]	8.9 (8.5, 9.3)		9.8 (9.4, 10.2)	
		<0.0001		<0.0001
Serum vitamin B-12 (pmol/L)[6]				
<256.8 (n = 1633)	11.9 (11.5, 12.3)[8]		11.9 (11.6, 12.3)[8]	
256.8 to <337.9 (n = 1622)	9.7 (9.3, 10.1)[8]		10.3 (9.9, 10.6)[8]	
337.9 to <444.2 (n = 1648)	9.2 (8.8, 9.6)[8]		10.0 (9.6, 10.3)	
≥444.2 (n = 1642)[9]	8.5 (8.1, 8.9)		9.6 (9.2, 10.0)	
		<0.0001		<0.0001

[1] RBC, red blood cell.
[2] Analysis of covariance with age, sex, and race-ethnicity as independent variables and tHcy as the dependent variable.
[3] ANOVA adjusted for age, sex, race-ethnicity, BMI, systolic and diastolic blood pressures, serum creatinine, alcohol intake, serum cotinine, supplement use, serum folate, RBC folate, and vitamin B-12.
[4] Effect of the age-, sex-, and race-ethnicity–adjusted variable on tHcy (analysis of covariance).
[5] Effect of the multivariate-adjusted variable on tHcy (multivariate ANOVA).
[6] Categorized by quartile.
[7] $\bar{x}$; 95% CI in parentheses.
[8] Significantly different from the reference group within the variable, $P < 0.05$ (Tukey's studentized pairwise comparison test).
[9] Used as a reference group for comparison within the categories of the variable.

nicotine is directly related to the serum cotinine concentration. Serum cotinine is a better indicator of smoking than is self-reported information because individuals tend to underreport cigarette smoking (41), and different persons inhale cigarette smoke differently (40). Also, serum cotinine reflects passive smoking. Serum cotinine concentration was correlated with number of cigarettes smoked in the NHANES III (29). By using serum cotinine rather than number of cigarettes smoked as a measure of smoking, we were able to assess the association between biochemical smoke and serum tHcy. The exact mechanism by which cigarette smoking increases tHcy is not known. However, the association between smoking and tHcy can be explained by low concentrations of blood folate, RBC folate (42), vitamin B-12 (43), and vitamin B-6 (44) in smokers.

In this study, alcohol consumption was a significant predictor of serum tHcy. Subjects who drank 1–30 drinks/mo had tHcy concentrations that were similar to those of subjects who drank no alcohol, suggesting that alcohol consumption of ≤1 drink/d may not adversely influence serum tHcy. We also found that hard-liquor consumption but not beer or wine consumption was a significant predictor of tHcy. Previously reported associations between alcohol consumption and tHcy were inconsistent (10, 30, 32, 45, 46). In the Framingham Study, a modest positive association between alcohol consumption and tHcy concentration was observed (10). That study also reported, similar to our findings, that hard-liquor consumption but not beer consumption was a significant predictor of tHcy. In contrast to our observations, de Bree et al (30) and Mayer et al (46) found an inverse association between beer consumption and tHcy. In acute alcohol intoxication, acetaldehyde, a metabolite of alcohol metabolism, exerts an inhibitory effect on methionine synthase (EC 2.1.1.13) (47). Methionine synthase is essential for the remethylation of homocysteine to methionine (11). In chronic alcoholics, increased tHcy can be explained by low circulating concentrations of folate, vitamin B-12, and vitamin B-6 (48).

The association between blood pressure and tHcy concentration suggests that subjects with systolic blood pressure ≥134 mm Hg had ≈3.1 μmol/L higher tHcy concentrations on average than did individuals with systolic blood pressure <110 mm Hg. This positive association between systolic blood pressure and tHcy confirms the findings of the Hordaland Study (3). Brattstrom et al (49) also reported a positive association between blood pressure and tHcy, but the association no longer persisted after adjustment for confounding variables. Others reported no association between systolic blood pressure and tHcy (10).

In conclusion, we confirmed the association between serum tHcy and various demographic characteristics, health and lifestyle factors, and blood concentrations of vitamins by using data from a nationally representative survey. Also, we reported that serum tHcy was inversely associated with RBC folate concentration and was positively associated with serum cotinine concentration. The variables sex and supplement use were not independently

associated with tHcy when other variables were considered. Because this study used cross-sectional data, it was not possible to evaluate the data in terms of cause-and-effect relations. Also, the data used in this study were collected before folate enrichment became mandatory in the United States in 1998. Because folate is an established predictor of tHcy concentration, it would be useful to investigate the effect of folate enrichment of foods on tHcy concentration in the current US population. Further studies are needed to establish the determinants of tHcy in various ethnic populations and in populations at risk for heart disease.

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