SERUM TOTAL HOMOCYSTEINE CONCENTRATION DETERMINANTS IN NON-HISPANIC WHITE, NON-HISPANIC BLACK, AND MEXICAN-AMERICAN POPULATIONS OF THE UNITED STATES

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Objective: Blood vitamins and the association between serum total homocysteine (tHcy) concentration and demographic, socioeconomic, health and lifestyle factors were investigated in non-Hispanic Whites (NHW), non-Hispanic Blacks (NHB), and Mexican Americans (MA).

Design and Setting: Cross-sectional data from the third National Health and Nutrition Examination Survey, 1988–1994, was used.

Participants: The study included 2,258 NHW, 1,856 NHB, and 1,584 MA.

Main Outcome Measures: Relationship between serum tHcy (dependant variable), and sex, age, income, education, alcohol consumption, vitamin/mineral supplement and medicine use, body mass index (BMI), systolic and diastolic blood pressures, serum creatinine, cotinine (a measure of smoking), folate and cobalamin, and red blood cell (RBC) folate (independent variables) was analyzed with multivariate analysis of covariance and linear regression.

Results: Serum tHcy was significantly higher in NHW and NHB than in MA. Serum and RBC folate were significantly higher and serum cobalamin was significantly lower in NHW compared to their counterparts. Serum folate and serum creatinine were the strongest determinants of tHcy in NHB and MA, and in NHW, respectively. The BMI was negatively associated with tHcy in NHB ($P=.02$) and in MA ($P=.002$) but not in NHW. Systolic blood pressure and serum cotinine were positively associated with tHcy only in NHW and MA. Education, income, supplement and medicine use, and alcohol consumption were not associated with tHcy concentration in any race-ethnicities.

Conclusions: In this large population based study, regardless of race-ethnicity, age, serum creatinine, folate, and cobalamin, and RBC folate were the major determinants of serum tHcy. (*Ethn Dis.* 2004;14:476–482)

Key Words: Alcohol, Folate, Homocysteine, Mexican American, National Health and Nutrition Examination Survey, NHANES III, Non-Hispanic Black, Race-Ethnicity, Smoking, United States

INTRODUCTION

Homocysteine is a non-protein forming, sulfahydryl amino acid formed from the demethylation of methionine.[1] Elevated total homocysteine (tHcy) in blood is an independent risk factor for vascular diseases.[2,3] A 5 μmol/L increase in serum tHcy concentration was associated with an increased relative risk for cardiovascular disease by ~60% in men and by ~80% in women.[4] Several possible mechanisms through which homocysteine increases the risk for heart diseases include oxidation of low-density lipoproteins, toxic effects on endothelial cells, impaired platelet activity, and increased smooth muscle cell proliferation.[2,5–7]

Homocysteine is either remethylated to methionine or transsulfurated to cysteine. Folate, riboflavin, and cobalamin are needed for remethylation. Remethylation of homocysteine is catalyzed by methionine synthase, a cobalamin dependent enzyme.[8] In this reaction, N^5-methyltetrahydrofolate serves as a methyl donor. This N^5-methyltetrahydrofolate is synthesized from N^5, N^{10}-methylenetetrahydrofolate by the action of methylenetetrahydrofolate reductase (MTHFR),[9] a riboflavin dependent enzyme.[10] Pyridoxine is required for transsulfuration of homocysteine to cysteine.[11,12]

Decreased circulating serum concentrations of folate, cobalamin, riboflavin, and pyridoxine are associated with increased serum tHcy concentration.[11,13,14] Serum creatinine and smoking are positively associated with serum tHcy.[11,13,15] The relationship between alcohol consumption and tHcy is inconsistent, and the association between body mass index (BMI) and tHcy has received little attention. Our previous analysis using the data from the third National Health and Nutrition Examination Survey 1988–1994 (NHANES III) confirmed race-ethnicity was a significant determinant of serum tHcy concentrations.[13] To our knowledge, determinants of serum tHcy in non-Hispanic Blacks (NHB) and Mexican Americans (MA) have never been studied. Therefore, the aim of this study was to analyze the associations of serum tHcy concentration with demographic, socioeconomic, health and lifestyle factors, and blood vitamins in non-Hispanic Whites (NHW), NHB, and MA using the data from a nationally representative survey of US residents.

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METHODS

Study Sample

The NHANES III was a multistage, stratified probability survey of non-institutionalized US residents conducted in 2 phases from 1988 to 1994. The

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current report is based on databases released for public use by the National Technical Information Service, Springfield, Va.[16–19] The detailed description of the survey methodologies was described elsewhere.[20] NHANES III sample consisted of 39,695 subjects. Only subjects with age ≥17 y (*N*=20,050) were included in the study because alcohol intake data was not collected for individuals <17 y. Participants with diabetes (*N*=1,614), and pregnant (*N*=338) and lactating (*N*=100) women were excluded from the study. Individuals with other race-ethnicities were excluded from the analysis due to small sample size (*N*=324). We excluded participants aged 90 years or higher (*N*=196) from the analysis because all individuals ≥90 y were categorized as 90 y+ to protect the confidentiality of participants. Subjects with missing data for tHcy and other covariates were also excluded (*N*=11,809). Thus, the current study was based on 5,669 subjects (NHW, 2,236; NHB, 1,852; and MA, 1,581).

Measurements

Depending on the age of the participant, data were collected on anthropometric measurements, demography, physical function, health condition, lifestyle behaviors, biochemical measurements of blood and urine, and diet intake. Blood was collected from venipuncture after fasting for varied lengths of time. Of 5,669 participants, 662 fasted for <6 h, 1,821 fasted for 6–10 h, and 3,186 fasted for >10 h. Length of fasting had no measurable effect on serum tHcy.[21] Serum tHcy concentrations were measured in surplus sera in phase 2 of the NHANES III (1991–1994) at the US Department of Agriculture Human Nutrition Research Center on Aging after the study protocol was approved by the New England Medical Center Human Investigations Review Committee. Serum tHcy was measured with the reversed-phase HPLC method.[22] Serum and red blood cell (RBC) folate, and serum cobalamin were measured at the Center for Disease Control (CDC) laboratories using radioassay methods.[23]

Blood pressure was measured using a mercury sphygmomanometer (W A Baum Co, Inc, Copiague, NY) according to the standard protocol recommended by the American Heart Association.[24] Blood pressure data is the mean of 6 or fewer measurements obtained at the household interview (maximum of 3) and at the mobile examination centers (maximum of 3). Participants were asked to report their consumption of beer (lite beer included), wine (wine coolers, sangria and champagne included), and hard liquor (gin, rum, whisky, tequila, vodka, liqueurs, etc) "times/month." One drink of alcohol was described as 12 oz of beer, 4 oz of wine, and 1 oz of hard liquor. Total number of alcoholic drinks consumed was computed by adding the number of drinks of beer, wine, and hard liquor. Participants who reported ≥1 total alcoholic drinks/mo were categorized as alcohol drinkers. Alcohol drinkers were further classified as light drinkers, moderate, and heavy drinkers if their reported consumption of total alcohol was 1–30 drinks/mo (up to 1 drink/d) and 31–60 drinks/mo (1–2 drinks/d), and ≥61 drinks/d (>2 drinks/d), respectively. Due to small sample size in the heavy drinker category, we collapsed moderate and heavy drinkers into one category (≥31 drinks/d).

We used serum cotinine concentration as a measure of intensity of smoking.[25] Serum cotinine was measured at the CDC laboratories using an enzyme-linked immunoassay.[23] In the NHANES III, serum cotinine concentration correlated with the number of cigarettes smoked.[26] Serum creatinine was analyzed as part of the 22 biochemical analytes at a CDC contracted laboratory (White Sands Research Center, Alamogordo, NH) using a multichannel analyzer (Boehringer Mannhelm Diagnostics, Indianapolis, Ind). Participants who answered "yes" to the question "Have you taken vitamins/mineral supplements in past month" were regarded as supplement users (*N*=2,166). Participants who answered 'yes' to the question "Have you taken prescription medicine in past one month" were regarded as medicine users (*N*=2,373). This population covers individuals who may be taking medications for various ailments such as thyroid, cancer, heart, hepatic, renal, pulmonary, digestive, and gout, which are known to affect homocysteine concentration. Education level was classified into 3 categories, ie, ≤8 y, 9–12 y, and ≥13 y. Participants income was classified into 4 categories, ie, ≤$9,999, $10,000–$19,999, $20,000–$39,000, and ≥$40,000. We selected sex, age, level of education and income, alcohol consumption, vitamin/mineral supplement and prescription medicine use, serum cotinine, body mass index (BMI), systolic and diastolic blood pressures, serum creatinine, serum folate, RBC folate, and serum cobalamin as potential determinants of serum tHcy concentration.

Statistics

Statistical analyses were performed with SUDAAN statistical software (SUDAAN, Release 8.0.2, Research Triangle Institute, Research Triangle Park, NC) in conjunction with SAS (SAS for Win-

Table 1. Selected characteristics of non-Hispanic Whites (NHW), non-Hispanic Blacks (NHB), and Mexican Americans (MA)*

Characteristic	NHW† (*N*=2,236)	NHB† (*N*=1,852)	MA† (*N*=1,581)	*P* Value‡ NHW vs NHB	NHW vs MA	NHB vs MA
Age, *y*	40.7 ± 0.4	35.9 ± 0.3	33.2 ± 0.4	<.001	<.001	<.001
Serum total homocysteine, *μmol/L*	9.2 ± 0.1	9.0 ± 0.1	8.4 ± 0.1	NS	<.001	.001
Alcohol intake, *drinks/mo*						
Total	8.6 ± 0.4	9.0 ± 0.4	6.7 ± 0.3	NS	<.001	<.001
Beer	6.4 ± 0.3	7.4 ± 0.4	6.0 ± 0.3	NS	NS	NS
Wine	3.4 ± 0.2	3.0 ± 0.2	2.6 ± 0.2	<.001	<.001	<.05
Hard liquor	3.6 ± 0.2	3.7 ± 0.2	2.3 ± 0.1	NS	<.001	<.001
Serum cotinine, *μg/L*	1.4 ± 0.1	3.4 ± 0.3	0.6 ± 0.1	<.001	<.001	<.001
Body mass index, *kg/m × m*	25.8 ± 0.1	27.1 ± 0.2	26.6 ± 0.1	<.001	<.001	<.003
Systolic blood pressure, *mm Hg*	120.4 ± 0.4	121.6 ± 0.4	117.5 ± 0.4	<.02	<.001	<.001
Diastolic blood pressure, *mm Hg*	74.0 ± 0.3	75.0 ± 0.3	72.2 ± 0.3	<.002	<.001	<.001
Serum creatinine, *μmol/L*	94.0 ± 0.4	97.7 ± 0.5	87.7 ± 0.4	<.001	<.001	<.001
Serum folate, *nmol/L*	13.4 ± 0.2	10.1 ± 0.1	10.9 ± 0.2	<.001	<.001	.03
Red blood cell folate, *nmol/L*	423.8 ± 4.9	295.6 ± 3.3	362.2 ± 4.2	<.001	<.001	<.001
Serum cobalamin, *pmol/L*	307.5 ± 2.9	379.8 ± 4.0	342.4 ± 4.6	<.001	.005	NS

* Weighted sample size (*N*): NHW, 50,239,644; NHB, 7,205,716; MA, 3,504,783.
† Geometric mean ± standard error.
‡ Pairwise comparisons with 2-tailed *t* test; NS, non-significant.

dows, Version 8.0, Cary, NC), which takes complex, stratified sample design into consideration. Means and standard errors were estimated using sample weights. Use of sample weights takes unequal probability of selection and non-response bias into consideration. Alcohol consumption, BMI, serum cotinine, serum creatinine, systolic and diastolic blood pressures, serum folate, RBC folate, and serum cobalamin were reported as continuous variables; level of education, income level and use of vitamin/mineral supplements and prescription medicines were reported as discrete variables in the NHANES III. The study sample was categorized into 4 age groups, ie, <30 y, 30–<50 y, 50–<70 y and 70–<90 y.

All continuous variables, except alcohol consumption, were divided into quartile cutoff points. Multivariate analysis of covariance (ANACOVA) was used to determine the adjusted means for serum tHcy within the levels of various determinants of serum tHcy. Pairwise mean comparisons were performed within the levels of determinants of serum tHcy with a two-tailed *t* test. Also, multivariate linear regression was used to evaluate the simultaneous effects of selected determinants of tHcy on serum tHcy. Since the data for serum tHcy concentration was skewed to the right, we performed regression analysis on log transformed tHcy. In the linear regression model, log tHcy was used as a dependent variable and BMI, alcohol consumption, serum cotinine, creatinine, folate and cobalamin, systolic and diastolic blood pressures, and RBC folate were used as independent variables adjusted for sex and age. Statistical significance (*P*) for linear trend and regression coefficients (β) were determined for independent variables. Statistical significance was set at $P<.05$.

RESULTS

The current study consisted of ~39% NHW, ~33% NHB, and ~28% MA. Selected characteristics of study participants by race-ethnicity are presented in Table 1. Due to the asymmetric nature of the data distribution, geometric means ± standard errors were presented on subjects characteristics. The mean age of NHW was significantly higher than NHB and MA. Serum tHcy in MA was significantly lower than NHW and NHB. Serum tHcy was ~9.5% and ~7.1% higher in NHW and NHB, respectively, compared to tHcy in MA. There was no significant difference in tHcy between NHW and NHB. On the average, NHB consumed the most amount of alcohol (~9.0 drinks/mo); MA consumed the least amount of alcohol (~6.7 drinks/mo). Beer was the most popular alcoholic beverage consumed by subjects. Wine consumption was significantly higher in NHW compared to other race-ethnicities examined. Serum cotinine, BMI, systolic blood pressure, and serum creatinine were significantly higher in NHB than those in NHW and in MA. Serum and RBC folate were significantly higher and serum cobalamin was significantly lower in NHW than in NHB and in MA.

Multivariate adjusted means for serum tHcy in NHW, NHB, and MA by demographic, lifestyle and health factors, and blood vitamins are presented in Table 2. Sex and age adjusted regression analyses of log tHcy concentration with independent variables are present-

Table 2. Multivariate adjusted serum total homocysteine (tHcy) concentrations in non-Hispanic whites (NHW), non-Hispanic Blacks (NHB), and Mexican-Americans (MA) by demographic, lifestyle and health factors, and blood vitamins*†

	Serum tHcy Concentration, μmol/L‡§		
	NHW (*N*=2,236)‖	**NHB (*N*=1,852)‖**	**MA (*N*=1,581)‖**
Sex			
Male	10.2 ± 0.2 (940)	9.8 ± 0.2 (821)	9.3 ± 0.2[a] (828)
Female	9.7 ± 0.2 (1,296)	9.7 ± 0.2 (1,031)	8.8 ± 0.2[b] (753)
P value#	.20	.79	.04
Age, *y*			
<30	9.4 ± 0.3[a] (387)	8.9 ± 0.2[a] (598)	8.6 ± 0.2[a] (564)
30–<50	9.5 ± 0.2[a] (678)	9.4 ± 0.2[b] (813)	8.9 ± 0.2[a] (621)
50–<70	10.5 ± 0.2[b] (613)	11.3 ± 0.4[b] (321)	10.4 ± 0.5[b] (296)
70–<90	12.0 ± 0.4[c] (558)	12.6 ± 0.5[c] (120)	12.2 ± 1.0[b] (100)
P value#	<.001	<.001	.001
Body mass index, *kg/m × m***			
Q 1	10.0 ± 0.2 (707)	10.0 ± 0.2[a] (506)	9.4 ± 0.2 (392)
Q 2	10.0 ± 0.2 (531)	10.2 ± 0.3[a] (363)	9.6 ± 0.3 (367)
Q 3	10.2 ± 0.3 (513)	9.3 ± 0.2[b] (412)	8.7 ± 0.2 (419)
Q 4	9.6 ± 0.2 (485)	9.3 ± 0.2[b] (571)	8.7 ± 0.3 (403)
P value#	.26	.02	.06
Alcohol consumption, *drinks/mo*			
0	9.8 ± 0.2 (1,142)	9.6 ± 0.2 (967)	8.8 ± 0.2 (783)
1–30	10.0 ± 0.2 (930)	9.7 ± 0.2 (757)	9.3 ± 0.2 (730)
≥31	10.5 ± 0.4 (164)	10.5 ± 0.5 (128)	9.4 ± 0.6 (68)
P value#	.27	.26	.39
Serum creatinine, *μmol/L***			
Q 1	8.6 ± 0.2[a] (200)	8.4 ± 0.2[a] (115)	8.0 ± 0.2[a] (317)
Q 2	9.9 ± 0.3[b] (387)	9.1 ± 0.2[b] (307)	8.5 ± 0.2[a] (334)
Q 3	10.0 ± 0.2[b] (525)	9.4 ± 0.3[b] (372)	9.2 ± 0.3[b] (315)
Q 4	11.0 ± 0.3[c] (1,124)	10.8 ± 0.3[c] (1,058)	9.9 ± 0.3[b] (615)
P value#	<.001	<.001	<.001
Serum folate, *nmol/L***			
Q 1	12.3 ± 0.4[a] (363)	11.8 ± 0.4[a] (532)	10.9 ± 0.4[a] (393)
Q 2	10.1 ± 0.2[b] (379)	9.7 ± 0.2[b] (470)	8.7 ± 0.2[b] (419)
Q 3	9.1 ± 0.2[c] (486)	9.2 ± 0.2[b] (448)	8.5 ± 0.2[b] (381)
Q 4	8.5 ± 0.3[d] (1,008)	8.2 ± 0.2[c] (402)	8.4 ± 0.4[b] (388)
P value#	<.001	<.001	<.001
Red blood cell folate, *nmol/L***			
Q 1	10.8 ± 0.4[a] (382)	10.3 ± 0.3 (821)	9.6 ± 0.4 (421)
Q 2	9.7 ± 0.2[b] (360)	9.5 ± 0.2 (423)	9.2 ± 0.2 (375)
Q 3	9.6 ± 0.2[b] (511)	9.6 ± 0.3 (350)	9.0 ± 0.2 (405)
Q 4	9.7 ± 0.3[b] (983)	9.4 ± 0.3 (258)	8.6 ± 0.3 (380)
P value #	.02	.10	.35
Serum cobalamin, *pmol/L***			
Q 1	11.5 ± 0.3[a] (756)	10.3 ± 0.3[a] (314)	10.5 ± 0.4[a] (378)
Q 2	9.9 ± 0.2[b] (557)	9.9 ± 0.2[a] (374)	8.8 ± 0.2[b] (390)
Q 3	9.2 ± 0.2[c] (464)	9.7 ± 0.2[a] (446)	8.8 ± 0.2[b] (389)
Q 4	9.3 ± 0.2[c] (459)	8.9 ± 0.2[b] (718)	8.3 ± 0.2[c] (424)
P value#	<.001	<.001	<.001

* Weighted sample size (*N*): NHW, 50,239,644; NHB, 7,205,716; MA, 3,504,783.

† Least square mean ± standard error, and *N* in the parenthesis.

‡ Multivariate Analysis of Covariance (ANACOVA) for tHcy with sex, age, education, income, body mass index, systolic and diastolic blood pressures, alcohol consumption, vitamin/mineral supplement and prescription medicine use, serum cotinine, creatinine, folate and cobalamin, and red blood cell folate.

§ Effect of education, income, serum cotinine, systolic and diastolic blood pressures, and use of vitamin/mineral supplement and prescription medicine was not significant in the multivariate ANACOVA in all race-ethnicities (data not shown).

‖ Means in a row with different superscripts [a,b,c] are significantly different at $P<.05$. Comparisons between 2 means were performed only in those variables that were significant in the ANACOVA. Pairwise comparisons with 2-tailed *t* test.

P value for the effect of variable in the multivariate ANACOVA by race-ethnicity.

** Categorized by quartile cut off points.

Table 3. Multiple linear regression analysis of serum total homocysteine (tHcy) with continuous independent variables for non-Hispanic Whites (NHW), non-Hispanic Blacks (NHB), and Mexican Americans (MA)*†

	NHW (*N*=2,236)‡		NHB (*N*=1,852)‡		MA (*N*=1,581)‡	
	β + SE§	*P*‖	β + SE§	*P*‖	β + SE§	*P*‖
Body mass index, *kg/m* × *m*	0.001 ± 0.002	NS	−0.003 ± 0.001	.02	−0.006 ± 0.002	.002
Systolic blood pressure, *mm Hg*	0.002 ± 0.001	<.001	0.001 ± 0.001	NS	0.002 ± 0.001	.01
Serum creatinine, *μmol/L*	0.006 ± 0.001	<.001	0.003 ± 0.001	<.001	0.007 ± 0.001	<.001
Serum cotinine, *μg/L*	0.0002 ± 0.0001	.001	0.0001 ± 0.0000	NS	0.0004 ± 0.0002	.006
Serum folate, *nmol/L*	−0.005 ± 0.002	.002	−0.009 ± 0.001	<.001	−0.013 ± 0.002	<.001
Red blood cell folate, *nmol/L*	−0.0003 ± 0.0001	<.001	−0.0004 ± 0.0001	<.001	−0.0002 ± 0.0001	.01
Serum cobalamin, *pmol/L*	0.0001 ± 0.0001	NS	−0.0001 ± 0.0001	.02	−0.0000 ± 0.0000	<.001

* Weighted sample size (*N*): NHW, 50,239,644; NHB, 7,205,716; MA, 3,504,783.
† Linear regression analysis with log transformed serum tHcy concentration.
‡ Effect of diastolic blood pressure and alcohol consumption was not significant in all race-ethnicities (data not shown).
§ Regression coefficient ± standard error.
‖ Effect of age and sex adjusted variable in the regression model; NS, non-significant.

ed in Table 3. In this study, in all race-ethnicity subset populations, males had significantly higher serum tHcy compared to females (data not shown). However, when confounding variables were considered in the ANACOVA, sex was significantly associated with serum tHcy only in MA (*P*=.04). Age was significantly associated with serum tHcy in all race-ethnicities. Serum tHcy concentration in 70–<90 y old compared to <30 y old were ~2.6 μmol/L (~27.7%,) higher in NHW, ~3.7 μmol/L (~41.6%) higher in NHB, and ~3.6 μmol/L (~41.9%) higher in MA. When other covariates were considered in the ANACOVA, education, income, vitamin/mineral supplement and prescription medicine use, systolic and diastolic blood pressures, and serum cotinine were not associated with serum tHcy in all race-ethnicities (data not shown).

Although alcohol consumption was not significantly associated with tHcy, there was a tendency toward higher serum tHcy with higher alcohol intake in all race-ethnicities (Table 2). After adjusting for confounding variables, BMI was significantly inversely associated with serum tHcy in NHB (*P* for linear trend =.02; β=−0.003) and in MA (*P* for linear trend =.002; β=−0.006) but not in NHW (*P* for linear trend =.71; β=0.001) (Table 3). Although no effect of systolic blood pressure on serum tHcy was found in the multivariate ANACOVA, a linear association was observed in the regression analysis in NHW (*P* for linear trend =.001; β=0.002) and in MA (*P* for linear trend =.01; β=0.002) but not in NHB (*P* for linear trend =.49; β=0.001). Similarly, multivariate adjusted serum cotinine was positively associated with serum tHcy in NHW (*P* for linear trend =.001; β=0.0002), and in MA (*P* for linear trend =.006; β=0.0004) but not in NHB (*P* for linear trend =.10; β=0.0001) (Table 3). Participants in the highest quartile compared to lowest quartile of serum creatinine had ~27.9% (~2.4 μmol/L) higher tHcy in NHW, ~28.6% (~2.4 μmol/L) higher tHcy in NHB, and ~21.3% (~1.9 μmol/L) higher serum tHcy in MA.

A negative association was observed between serum folate and serum tHcy in NHW, NHB, and MA. In the lowest serum folate quartile group compared to the highest serum folate group, serum tHcy concentration was ~3.8 μmol/L higher in NHW, ~3.6 μmol/L higher in NHB, and ~2.5 μmol/L higher in MA. In regression analysis, a weak negative association was observed between RBC folate and tHcy. When covariates were considered in the multivariate analysis, RBC folate was significantly associated with serum tHcy in NHW (*P*=.02) but not in NHB (*P*=.10) and MA (*P*=.35). In all race-ethnicities, serum cobalamin was significantly related to serum tHcy (Table 2).

DISCUSSION

This is the first study that reports the determinants of serum tHcy concentration in NHW, NHB, and MA using the data from a nationally representative sample survey of the US residents. Previously, using the data from the NHANES III, Jacques et al[21] and Selhub et al[27] reported race-ethnicity and gender specific differences in serum tHcy. Since serum tHcy concentrations are influenced by several demographic, lifestyle, genetic, and nutritional factors, it is important to investigate serum tHcy determinants separately in various race-ethnicities. In this study, in all race-ethnicities, age, serum creatinine, folate, and cobalamin, and RBC folate were significant determinants of serum tHcy. These findings support previously reported associations by several investigators in various populations.[3,11,27,28] In our study, serum folate was the strongest determinant of serum tHcy in both NHB (*P* for linear trend =<.001; β=−0.009) and MA (*P* for linear trend =<.001; β=−0.013); serum creati-

In this study, in all race-ethnicities, age, serum creatinine, folate, and cobalamin, and RBC folate were significant determinants of serum tHcy.

nine was the strongest determinant of tHcy in NHW (P for linear trend =<.001; β=0.006). In all race-ethnicities, serum folate was a stronger determinant of tHcy than RBC folate. Additionally, we report income, education levels, and vitamin/mineral supplement use had no association with serum tHcy, suggesting socioeconomic status is not a determinant of serum tHcy.

Evidence of racial difference in serum tHcy was first reported by Ubbink et al.[29] They reported significantly higher serum tHcy in adult South African White men (N=1,437) than those of Black (Venda tribe) men (N=117). It was suggested that depressed tHcy in Black was due to more efficient homocysteine metabolism in Black than in White.[30] In contrast, we observed no difference in serum tHcy between NHW and NHB, suggesting homocysteine metabolism in NHB differs from those in South Africa.

Across the gender and age groups, MA had the lowest serum tHcy. This can be attributed to several factors. Serum cotinine in MA was ~133% (~0.8 μg/L) and ~467% (~2.8 μg/L) lower compared to serum cotinine in NHW and in NHB, respectively, suggesting lower incidence of smoking in MA than in NHW or NHB. Also, MA had significantly lower serum creatinine and higher serum cobalamin compared to their counterparts. All these aforementioned factors favor lower serum tHcy.[3,11,13] Additionally, the number of subjects in the 50–<90 y age group was much higher (~52%) in the NHW compared to NHB (~24%) and MA (~25%). Results from this study and from earlier studies clearly documented that the tHcy concentration are significantly higher in older persons than in younger persons. So, the higher serum tHcy concentration in NHW compared to NHB and MA may partly be explained by the increased sample size in the 50–<90 y age group of NHW.

We found alcohol was not a significant determinant of serum tHcy in all race-ethnicities, although results suggest a positive trend between serum tHcy and alcohol consumption. Earlier studies on association between alcohol consumption and circulating tHcy concentration yielded mixed results.[11,31,32] Some reported a positive association between alcohol consumption and serum tHcy.[11] Others reported no association between alcohol intake and plasma tHcy.[31] Interestingly, Mayer et al[32] found a negative association between beer consumption and tHcy. This was attributed to improved folate and vitamin B-12 status in beer drinkers because beer is an important source of these vitamins especially in populations with low folate intake.

We used serum cotinine as a measure of smoking rather than self-reported cigarette smoking because individuals tend to under report smoking.[33] Also, serum cotinine captures passive smoking. By using serum cotinine, we were able to analyze the association between biochemical smoke and serum tHcy. Serum cotinine was a significant determinant of tHcy in all race-ethnicities except NHB (regression analysis) suggesting more adverse effect of smoking in NHW and MA than in NHB. However, the exact mechanism through which cotinine influences tHcy concentration is not known.

Serum tHcy was negatively associated with BMI in NHB and in MA but not in NHW. This observation is rather surprising and unexplainable. The association between BMI and serum tHcy needs further investigation. In this study, systolic blood pressure was a significant determinant of tHcy only in NHW and MA (regression analysis) and this association no longer persisted in the multivariate ANACOVA. Previously reported studies relating serum tHcy and systolic blood pressure has yielded mixed results.[3,11] In the Hordland study, a positive linear association was observed between tHcy and systolic blood pressure and this association was confined only to the younger group.[3] On the other hand, no association was observed between tHcy and blood pressure in the Framingham Study.[11]

It is important to note that the measurement of cause and effect relationship is not possible because the data was derived from a cross sectional study. Although medication which may increase the tHcy concentrations such as antiasthma, antidepressant, or anticonvulsive and diseases such as cancer, cadiomyopathy, gout, migraine, peptic ulcer, renal, thyroid, and hepatic may increase the tHcy concentration, we did not control our tHcy data for these conditions separately. However, the data was adjusted for the medication use (yes or no) in the last 30 days. Also, we were unable to adjust the tHcy for a specific vitamin supplement because often vitamin supplements contain more than one vitamin and thus makes it difficult to adjust the data for a specific vitamin. Alternatively, we adjusted the data for vitamin/mineral supplement use (yes or no) in last 30 days. In this study, we did not investigate the potential association between dietary intakes and tHcy because in the NHANES III the dietary intakes were quantified from one 24-hour diet recall. Food intakes based on one-day 24-hour recall tend to be unreliable. In this large population based study, although some differences existed in determinants of serum tHcy, regardless of race-ethnicity, age, serum creatinine, folate and cobalamin and RBC folate were the significant determinants of serum tHcy concentration. Although

speculative, the race-ethnicity differences in determinants of serum tHcy were more likely due to differences in genetic, lifestyle, and nutritional factors among NHB, MA, and NHW.

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Manuscript draft: Ganji
Statistical expertise: Kafai
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