SERUM HOMOCYSTEINE CONCENTRATIONS AND THEIR RELATION TO SERUM FOLATE AND VITAMIN B_{12} CONCENTRATIONS AND CORONARY ARTERY DISEASE PREVALENCE IN AN URBAN, BI-ETHNIC COMMUNITY

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Objective: To compare fasting serum total homocysteine (tHcy) concentrations in a randomly selected sample of elderly (≥65 years of age) Hispanic and non-Hispanic White (NHW) men and women, to examine associations of tHcy with folate and vitamin B_{12}, and then to correlate these with the prevalence of coronary heart disease (CHD) in these 4 ethnic/gender groups.

Design and Participants: Equal numbers of Hispanic and NHW men and women were randomly selected from the Healthcare Financing Administration (Medicare) registrant list for Bernalillo County (Albuquerque), New Mexico, and asked to volunteer for a paid home interview, to be followed by a paid, comprehensive interview/examination covering health and health-related issues.

Interventions and Main Outcome Measures: Serum concentrations of tHcy, folate, and vitamin B_{12} were determined and correlated with the prevalence of CHD, after adjusting for other CHD risk factors (age, diabetes, hypertension, smoking, dyslipidemia, adiposity).

Results: Men and Hispanics had higher serum tHcy concentrations compared to women and non-Hispanic Whites (NHWs), respectively. After adjusting for lower concentrations of serum folate and vitamin B_{12} in Hispanics, the differences between Hispanics and NHWs were no longer significant. There was a direct association between serum tHcy concentrations and the prevalence of CHD after adjusting for other known risk factors that was most significant in Hispanic women.

Conclusions: The higher serum tHcy concentrations observed in Hispanics compared to NHWs can be explained by lower levels of serum folate and vitamin B_{12}. A direct association between serum tHcy concentrations and prevalence of CHD was observed primarily in women, and was most significant in Hispanic women. (*Ethn Dis*. 2003;13:178–185)

Key Words: Homocysteine, Coronary Heart Disease, Folate, Vitamin B_{12}

INTRODUCTION

Both mildly and markedly elevated circulating total homocysteine (tHcy) concentrations are associated with increased risk of atherosclerotic vascular events (coronary heart disease, stroke, peripheral vascular disease).[1–4] It remains unclear whether elevated serum tHcy concentrations are actually a strong, independent risk factor for vascular disease, or are merely a marker for existing vascular disease.[5,6] Elevated fasting serum tHcy concentrations can be normalized by folic acid, vitamin B_{12}, and vitamin B_6 (pyridoxine) supplements.[7,8] Little information exists on ethnic differences in serum tHcy concentrations and their associations with either concentrations of these or coronary heart disease prevalence, especially in the elderly. Initial data from the third National Health and Nutrition Examination Survey (1991–1994) suggested that young Mexican-American men and women might have lower serum tHcy concentrations than either non-Hispanic Whites (NHW) or non-Hispanic Blacks (NHB), although the differences could have been explained by the younger mean age of the Mexican Americans, as serum tHcy levels increase with age.[9] A later analysis of this survey, using age-adjusted geometric means (log transformations) instead of arithmetic means (because the tHcy concentrations were extremely skewed) found that Mexican-American women, but not Mexican-American men, had lower ($P<.01$) serum tHcy concentrations compared to their NHW and NHB counterparts.[10] If a difference does exist, this might help to explain the lower prevalence of coronary heart disease (CHD) reported in Mexican Americans, as compared to non-Hispanic Whites (NHWs), despite a higher prevalence of such CHD risk factors as diabetes and obesity among NHWs.[11,12]

The New Mexico Elder Health Survey (NMEHS) was a study of health and health-related issues conducted between l993 and 1995 on nearly equal numbers of elderly (≥65 years of age) Hispanic and NHW men and women randomly selected from the Healthcare Financing Administration (HCFA) (Medicare) rolls of Bernalillo County (Albuquerque), New Mexico.[13–15] Hispanics had lower levels of serum vitamin

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Presented at the American College of Nutrition Annual meeting, Las Vegas, Nevada, October 14, 2000.

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B_{12} and folate than did NHWs, even after adjustments for gender, age, vitamin supplementation, vitamin content of dietary foods, and household income.[15] The purpose of this study is to compare serum tHcy concentrations in Hispanics and NHWs in the NMEHS, to examine their associations with serum folate and vitamin B_{12} concentrations, and then correlate these with the prevalence of CHD, after adjusting for the prevalence of other known CHD risk factors (age, diabetes, hypertension, smoking, dyslipidemia, adiposity).

METHODS

Study Design/Subjects

Twenty-two hundred prospective participants (equal number of Hispanic and NHW men and women) were randomly selected from the 50,700 HCFA registrants (Medicare recipients), aged 65 years and older, residing in Bernalillo County (Albuquerque), New Mexico. After eliminating those who had died, or had moved from the county, those who could not be located, either because a street address was unavailable, or they did not respond to notes left at their homes, and those who were ineligible because they clearly did not meet criteria to qualify as Hispanic or NHW, 1666 eligible participants were contacted. One thousand one hundred thirty (67.8%) agreed to participate in home interviews. An additional 29 interviewees were thereafter eliminated from the study because they either did not meet ethnicity standards (self-identification and 3 of 4 grandparents identified as Hispanic or NHW), or had died or moved during the time period following the the home interview, but prior to completion of an examination. Of the 1101 individuals interviewed at home and found to be eligible, 883 (80.2%) participated in a 4-hour interview/examination by a nurse practitioner, nurse, and nutritionist. Where informed consent could be obtained from the participant or a legal guardian, no other exclusion criteria were applied. A senior health center was used for 89% of the examinations, with the remainder being performed in residences or nursing homes. All participants gave written informed consent, and the research was approved by the Human Research Review Committee of the University of New Mexico Health Sciences Center. Further details on the design and survey instruments used in the study, and demographic characteristics of the recruited participants, are published elsewhere.[13,14]

Laboratory Determinations

Blood samples were drawn and immediately placed in ice until separation. Serum samples had been stored at −70 degrees Centigrade for periods of up to 3 years. Serum tHcy concentrations were determined using a procedure described by Stabler et al.[16,17] Total tHcy was extracted and partially purified from serum using reduction with dithiothreitol followed by anion exchange chromatography. The t-butyldimethylsilyl-derivatives were prepared and analyzed using capillary gas chromatography–mass spectrometry with selected ion monitoring. The methodology for the radioassays of vitamin B_{12} and folate have been discussed in detail in a previous publication.[15] Serum creatinine concentrations were determined by an Autoanalyzer as part of a SMAC-20 panel.

Coronary Heart Disease and Its Risk Factors

Participants were considered to have CHD if they met any one of 4 criteria, specifically: 1) a self-report of a physician's diagnosis of myocardial infarction or angina pectoris; 2) a history of coronary artery bypass graft (CABG) surgery; 3) the presence of a definite myocardial infarction on ECG by computer interpretation (MAC-VU, Marquette Electronics, Milwaukee, Wis) overread by a cardiologist (probable and possible infarctions were not included); and 4) a history of chest pain in the past 6 months with at least 2 of 5 qualifiers suggesting CHD (substernal in location; radiating to jaw, neck, or left arm; brought on by exercise; relieved by rest or nitroglycerin; associated with shortness of breath, nausea, vomiting, or sweating). Risk factors examined were age, presence of diabetes (undergoing treatment, or exhibiting either fasting serum glucose $\geq$126 mg/dL or 7.0 mmol/L, or 2-hour post-prandial glucose $\geq$200 mg/dL or 11.1 mmol/L), smoking history, current systolic and diastolic blood pressures (mm Hg), total and HDL-cholesterol concentrations (mg/dL), and total body adiposity, or body mass index, determined by body weight/height (kg/m^2). Further details on methodology, and on measures of the various CHD risk factors, are published elsewhere.[13]

Statistical Methods

Descriptive statistics include mean $\pm$ standard deviations and frequency rates (%), and division into quartiles. In making comparisons between group frequency rates, the chi-square test was used. In making comparisons between groups on continuous variables, and where normal distribution of the measure held, a *t* test was used. A logarithmic transformation of skewed variables (tHcy, folate, B_{12}), followed by a *t* test,

was used when approximate normal distribution could be obtained. Linear regression models were constructed to show the association between the log of serum tHcy concentrations as continuous variables using the logs of serum folate and/or vitamin B_{12} concentrations as predictors, and age and ethnicity as covariates for both genders. Linear regression models were also developed to show the associations between serum tHcy concentrations (as log of continuous variables), and prevalence of CHD (odds ratios, 95% confidence intervals, *P* values) adjusting for age, presence of diabetes, current smoking history, systolic and diastolic blood pressures, total and HDL-cholesterol concentrations, body mass index (BMI), and, when appropriate, gender and ethnicity. Logistic regression models were used to show the associations between serum tHcy concentrations (as dichotomous variables $\geq$15 vs $<$15 μmol/L) and prevalence of CHD, adjusting for the same variables. All analyses were performed using SAS software.[18]

Table 1. Fasting serum total homocysteine (tHcy) concentrations (means ± SD in μmol/L) by age categories for Hispanic and non-Hispanic White men and women in the New Mexico (NM) Elder Health Survey

	Age Groups		
	65–74 Years	75–84 Years	85+ Years
Hispanic men			
No. participants	132	49	17
Mean tHcy concentration*	14.3 ± 1.4	15.6 ± 1.4	16.6 ± 1.8
Non-Hispanic White men			
No. participants	136	69	17
Mean tHcy concentration	12.8 ± 1.3	14.0 ± 1.3	16.8 ± 1.4
Hispanic women			
No. participants	111	50	12
Mean tHcy concentration	12.4 ± 1.3	12.8 ± 1.4	14.7 ± 1.3
Non-Hispanic White women			
No. participants	113	71	14
Mean tHcy concentration	11.2 ± 1.3	12.6 ± 1.4	14.2 ± 1.2

* Logarithmic transformation with restoration to arithmetic means.

RESULTS

Stored serum samples were available for analyses of serum tHcy concentrations on 791 participants (mean age 74.1 years). Although the distribution of arithmetic means was skewed, log transformation normalized the distribution (geometric means). Geometric means were used for statistical analyses. The geometric (logarithmic transformation with restoration to arithmetic values) means ± standard deviations for serum tHcy concentrations for the 4 gender/ethnicity groups by age category are shown in Table 1. More Hispanic men (41.4%) than NHW men (33.3%) had serum tHcy concentrations $\geq$15 μmol/L, and more Hispanic women (27.2%) had high levels compared to NHW women (21.2%). This cut point was chosen arbitrarily because it represented the top tertile of values.

Men had higher mean tHcy concentrations compared to women (Beta=0.14, S.E.E.=0.02, *P*=.0001). Hispanic men and women both had significantly higher unadjusted mean fasting serum tHcy concentrations as compared to their NHW counterparts (Beta=0.09, S.E.E.=0.03, *P*=.004 for men; Beta=0.06, S.E.E.=0.03, *P*=.048 for women).

In a previous report from the NMEHS,[15] Hispanic participants had lower serum concentrations of folate (*P*<.001) and vitamin B_{12} (*P*<.05) than did NHWs, after adjusting for the effects of gender, age, vitamin supplementation, education, and annual household income. These lower serum vitamin levels were only partially explained by the fact that the Hispanics took vitamin supplements less frequently than did NHWs (32% vs 48%, respectively). Men had lower folate levels than did females (*P*<.05). Since others[7,9,19–22] have consistently demonstrated an inverse association between serum tHcy concentrations and serum concentrations of folate and vitamin B_{12}, it was hypothesized that the differences in serum tHcy concentrations between the 2 ethnic groups (and possibly the 2 genders) might be explained by differences in serum vitamin concentrations.

To explore this possibility, group comparisons were made for the 4 gender-by-ethnicity groups using a general linear model (GLM) to compare serum tHcy concentrations, using folate and vitamin B_{12} as predictors, and age as a covariate. Distributions of tHcy, folate, and B_{12} concentrations were skewed, so log transformations were used to normalize the distributions. The GLM suggests that the effects of gender and ethnicity are additive, rather than multiplicative (*P*=.29). The rates of change for log (tHcy), given either log (folate) or log (B_{12}), are the same for both ethnic groups and genders. When testing the 2 main effects of gender and ethnicity separately, the effect of gender was significant (*P*=.0001), but that of ethnicity was not (*P*=.70). These findings would seem to indicate that the differences in serum tHcy concentrations could be explained by differences in age, gender, folate, and B_{12} concentrations; however, there was no difference by ethnicity after adjusting for these variables. This model has an adjusted R^2 of 28%, indicating that important predictors are not included in this model.

Table 2. Summary statistics by quartiles of fasting serum homocysteine concentrations (mean ± SD and range), serum folate and vitamin B_{12} concentrations (mean ± SD), prevalence of CHD and selected risk factors (diabetes, smoking), and mean ± SD for other risk factors (systolic and diastolic blood pressures, total and HDL cholesterol concentrations, and body mass index) for male participants in the NMEHS

	Q1	Q2	Q3	Q4	*P**
Male					
No. participants	107	103	109	101	
Serum tHcy (μmol/L)	9.9 ± 1.2	12.2 ± 0.5	15.2 ± 1.2	23.2 ± 11.3	
(range)	5.7–11.3	11.3–13.2	13.2–17.0	7.0–102	
Serum folate (pmol/L)	15.5 ± 11.1	14.3 ± 10.5	9.7 ± 6.2	8.5 ± 10.3	<.0001
Serum B_{12} (nmol/L)	521 ± 202	489 ± 202	427 ± 173	413 ± 311	.0002
Age (years)	73.0 ± 5.3	73.0 ± 5.6	74.6 ± 6.0	75.0 ± 6.9	
Hispanics (%)	43.9	41.8	45.9	57.4	
CHD (%)	29.9	35.0	39.4	30.7	.899
Diabetes (%)	28.3	29.7	27.1	19.0	.065
Current smoker (%)	15.1	13.9	13.1	15.2	.907
Systolic pressure (mm Hg)	40.9+20.3	140.7+19.1	137.2+22.5	142.7+16.8	±.867†
Diastolic pressure (mm Hg)	77.4+9.8	80.0+10.1	77.2+10.1	78.4+10.6	±.416†
Total cholesterol (mg/dL)	198.9+41.2	199.2+33.5	199.9+42.8	189.1+46.7	±.732†
HDL-cholesterol (mg/dL)	44.0+13.6	43.2+12.1	42.1+10.8	44.7+16.8	±.404†
Body mass index (kg/m²)	25.8+3.6	25.3+3.8	26.2+3.6	26.1+4.1	±.228†
Female					
No. participants	93	97	90	91	
Serum tHcy (μmol/L)	8.6 ± 0.9	10.8 ± 0.7	13.4 ± 0.9	19.1 ± 6.6	
(range)	5.9–9.8	9.8–11.9	11.9–14.9	14.9–68.4	
Serum folate (pmol/L)	19.6 ± 13.6	17.4 ± 15.6	12.4 ± 11.7	9.1 ± 9.1	<.0001
Serum B_{12} (nmol/L)	623 ± 320	564 ± 276	465 ± 199	387 ± 188	<.0001
Age (years)	72.5 ± 5.3	73.4 ± 5.7	73.7 ± 6.8	75.8 ± 6.5	
Hispanics (%)	40.9	45.4	46.7	53.9	
CHD (%)	15.0	15.5	18.9	30.8	.011
Diabetes (%)	16.7	13.8	17.0	19.3	.342
Current smoker (%)	13.2	10.4	19.3	18.9	.047
Systolic pressure (mm Hg)	137.3+18.9	136.2+20.4	140.3+23.5	139.1+20.3	±.819†
Diastolic pressure (mm Hg)	71.4+10.8	71.5+10.5	72.2+11.8	73.4+10.5	±.351†
Total cholesterol (mg/dL)	224.6+43.4	220.6+36.5	214.3+39.3	219.8+42.0	±.903†
HDL-cholesterol (mg/dL)	54.8+14.5	51.6+13.7	55.3+14.9	50.5+14.0	±.088†
Body mass index (kg/m²)	25.8+4.6	26.3+5.0	26.4+4.7	26.5+5.0	±.100†

* *P* values for trends adjusted for age and ethnicity. † means ± SD

The model using folate alone as the predictor was then tested. Again, the effects of gender and ethnicity were found to be additive, and the rates of change in log (tHcy) given log (folate) are the same for the 4 gender-by-ethnicity groups. Log (tHcy) concentrations were significantly different between male and female groups ($P=.0001$), regardless of participants' ethnicity. The ethnicity effect is not significant for either gender ($P=.576$ for men; $P=.423$ for women) (see Figure 1 for a visual representation). These findings can be interpreted as an indication that the difference in serum tHcy concentrations observed between the 2 ethnicities can be explained by the difference in serum folate levels alone.

Significant associations between the serum fasting tHcy concentrations and the prevalence of CHD were also sought in this population, after adjusting for the presence of a number of known CHD risk factors. These risk factors were the presence of diabetes, current smoking history, systolic and diastolic blood pressures, total cholesterol and HDL-cholesterol concentrations, and BMI as continuous variables, and age, gender, and ethnicity as appropriate. The results of these linear regression models are shown in Table 3. Serum tHcy concentrations are shown both as the log of continuous variables, and as a dichotomous variable (≥15 μmol/L vs <15 μmol/L). When all participants are included in the model, the odds ratio for the association between tHcy levels as the log of the continuous variable and the prevalence of CHD is marginally significant ($P=.087$). When the genders are analyzed separately, the association for men is not significant, but the odds ratio is significant for women ($P=.008$). Similarly, when the Hispanic women and NHW women are modeled separately, the Hispanic women have an odds ratio that is significant ($P=.03$),

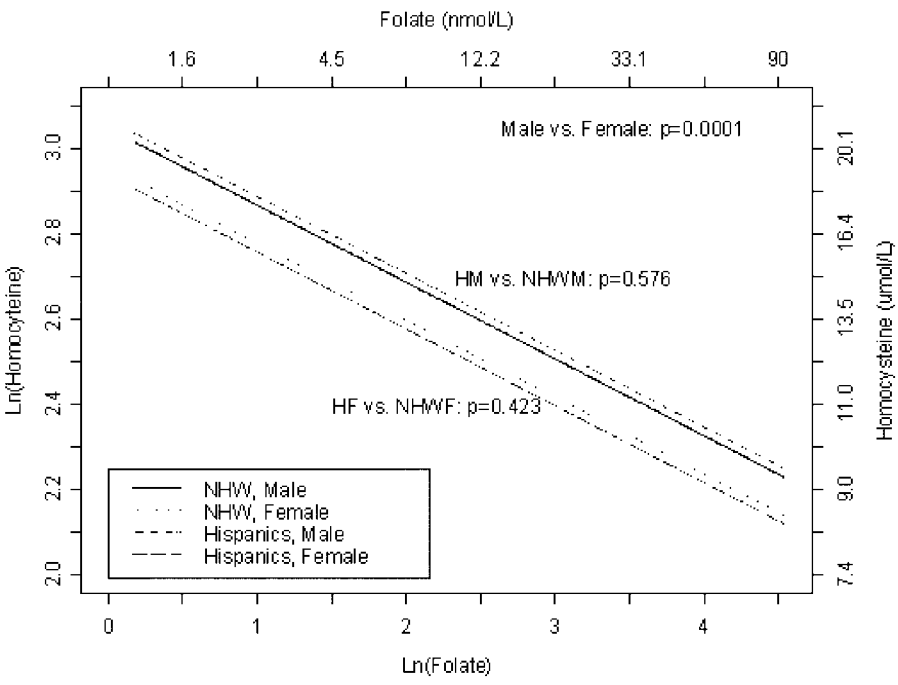

Fig 1. Predicted association between fasting serum total homocysteine and folate concentrations (log values) in the 4 ethnicity/gender groups in the New Mexico (NM) Elder Health Survey

but the NHW women did not show a significant association. When the associations between serum tHcy concentrations as a dichotomous variable (≥15 μmol/L vs <15 μmol/L) and the prevalence of CHD are subjected to similar analyses using logistic regression analyses, similar odds ratios are observed.

The level of renal function is another variable that can affect serum tHcy concentrations. Hispanic and NHW men have similar mean ± standard deviations of serum creatinine concentrations (125 ± 45 vs 126 ± 23 μmol/L, respectively). This similarity also exists for the Hispanic and NHW women (97 ± 20 vs 101 ± 20 μmol/L). The Modification of Diet in Renal Disease Study Group has proposed the use of a formula (Formula 7) incorporating serum creatinine, age, gender, ethnicity, and serum urea nitrogen and albumin concentrations corrected to a body surface area of 1.73 m^2 (dependent on height and weight) to estimate glomerular filtration rates.[19] The calculated glomerular filtration rates (mean ± SD) are 64.4 ± 16.3 ml/min/1.73 m^2 for Hispanic men vs 57.8 ± 13.0 ml/min/1.73 m^2 for NHW men, and 71.9 ± 15.8 ml/min/1.73 m^2 for Hispanic women vs 64.0 ± 14.4 ml/min/1.73 m^2 for NHW women. Since both Hispanic men and women have higher glomerular filtration rates compared to their NHW counterparts ($P<.001$), lower levels of renal function in Hispanics would not appear to explain the higher concentrations in serum tHcy concentrations observed in this ethnic group.

Table 3. Odds ratios (95% confidence intervals) and *P* values showing associations between fasting serum homocysteine concentrations and prevalence of CHD in the New Mexico Elder Health Survey (NMEHS)

Homocysteine Concentration	Continuous Variable[a] (per one log unit in μmol/L)		Dichotomous Variable (≥15 vs <15 μmol/L)	
	OR (95% CI)	*P*	OR (95% CI)	*P*
All participants	1.59 (0.94–2.69)	.087	1.38 (0.97–1.98)	.077
Men	1.08 (0.55–2.13)	.826	1.02 (0.65–1.59)	.944
Women	3.52 (1.16–7.22)	.008	2.99 (1.59–5.62)	.001
Hispanic men	0.87 (0.35–2.12)	.754	1.03 (0.54–1.95)	934
NHW men[b]	1.51 (0.50–4.55)	.466	1.00 (0.53–1.92)	.990
Hispanic women	4.61 (1.16–18.4)	.030	4.30 (1.74–10.6)	.002
NHW women	2.93 (0.80–10.8)	.105	1.67 (0.64–4.34)	.296

[a] Adjusted for diabetes (+/−), current smoking history (+/−), systolic and diastolic blood pressures (mm Hg), total and HDL cholesterol concentrations (mg/dL), body mass index (kg/m^2), and age (years), and gender and ethnicity as appropriate.

[b] NHW=non-Hispanic White.

DISCUSSION

In this population-based study of a randomly selected sample of predominantly community-dwelling elders (≥65 years of age), higher fasting serum tHcy concentrations were observed in Hispanic participants, when compared to NHW participants of comparable age. The inverse associations of serum tHcy with serum folate and/or vitamin B_{12} were similar for elderly Hispanic and NHW men and women, and the ethnic difference in serum tHcy concentrations was lost in models adjusted for serum folate and/or vitamin B_{12}. In models adjusted for age and major CHD risk factors, a marginally significant ($P=.087$) positive association was found between fasting serum tHcy concentration as log of a continuous variable and prevalence of CHD (OR=1.59). This association was largely attributable to a significant association among all women (OR=3.52, $P=.008$) that was stronger among Hispanic women (OR=4.61, $P=.03$).

Selhub et al,[9] reporting on findings

from the third National Health and Nutrition Examination Survey (NHANES III) (1991–1994), found that young Mexican-American (MA) men and women (mean age of 33 years) had lower serum tHcy and folate concentrations compared to their NHW counterparts. The Mexican Americans (MAs), however, also had a mean age almost 10 years younger than that of the NHWs, and serum tHcy concentrations rose significantly as age increased.[9] A subsequent report from this study, showing age-adjusted geometric means for specific age groups, compared MAs with NHWs and non-Hispanic Blacks (NHBs).[10] These investigators found the men to have significantly higher mean serum tHcy levels than females in each race-ethnicity group ($P<.01$), with a progressive increase in tHcy levels with age. There was a significant age-sex interaction ($P=.01$), reflecting the fact that women's tHcy concentrations tended to diverge from those of males at younger ages, and then converge with those of males at older ages. While MA women had significantly lower ($P<.01$) serum tHcy levels compared to the other 2 groups, no significant differences were observed in the 3 male race-ethnicity groups.

Selhub et al[7,9,10] and others[20–23] also have found a strong inverse association between serum tHcy levels, and serum folate, and, to a lesser extent, vitamin B_{12} concentrations. A previous report from the current study showed that serum folate and vitamin B_{12} concentrations were lower in the Hispanic population compared to the NHW population, and that this was only partially explained by the fact that the Hispanic participants took multivitamin supplements (32%) less frequently than did the NHW (48%).[15] Therefore, it was not surprising to find that the Hispanic participants, essentially the same age as the NHW participants, had higher serum tHcy concentrations. The higher serum tHcy concentrations observed in Hispanics appear to be explained solely by the lower folate and/or vitamin B_{12} concentrations.

Selhub et al,[7,9] using larger populations, demonstrated that an inverse curvilinear relationship exists between serum total homocysteine and folate (and B_{12}) concentrations, with the slope of serum tHcy concentrations increasing more steeply at lower serum folate levels. A similar relationship was found in the current study, plotting log-linear regression lines using logarithmic transformations of tHcy, folate, and B_{12}. No differences in the relationships between serum tHcy levels, folate, and/or B_{12} concentrations were observed between the Hispanic and NHW participants. Selhub et al[7,9,10] also found women (compared to men) to have lower serum tHcy levels for any given folate or B_{12} level.

On first examination, the serum tHcy levels reported in the current study appear high. This may be explained by the fact that few studies have reported on a population as old as that of the current study (mean age 74.1 years). Fasting serum tHcy concentrations clearly increase progressively with age,[9,10,20–23] and the current findings are very similar to the tHcy levels reported by Bates et al[21] in their study with older participants (75+ years). The study from NHANES III, as reported by Jacques et al,[10] demonstrated slightly lower mean values than the current study, when comparing geometric means for comparable age groups.

In a comprehensive review of recent epidemiologic studies, Eikelboom et al[24] reported that elevated levels of serum tHcy are prevalent in the general population, and are associated with an increased risk of cardiovascular disease, independent of the classic cardiovascular risk factors (age, diabetes, hypertension, smoking, dyslipidemia, and adiposity). The European Concerted Action Project (ECAP), a case-control study conducted in 19 centers of 9 European countries, and designed to examine the extent to which an elevated plasma tHcy concentration increases risk for vascular disease, found that an elevated plasma tHcy concentration conferred an independent risk similar to that of smoking and hyperlipidemia.[1] Eikelboom et al[24] also summarized previous reports demonstrating that simple, non-expensive therapy with folic acid and vitamins B_{12} and B_6 reduces plasma tHcy concentrations. Although the association between serum or plasma tHcy concentrations and CHD is strong and biologically plausible, it does not prove the existence of a causal relation, as recently debated by Brattstrom and Wilcken[5] and Ueland et al.[6] The fact that Hispanic participants did not exhibit lower serum tHcy concentrations than those of the NHW participants, appears to invalidate our initial hypothesis that the lower prevalence of CHD in the Hispanic population[11,12] might be associated with lower levels of tHcy.

Serum tHcy concentrations were directly associated with CHD prevalence in women, especially Hispanic women, but no association was observed in the men in the current survey. Little information was found in the literature comparing the associations between elevated serum tHcy concentrations and prevalence of CHD in different race/ethnicity groups. Folsom et al[25] reported a similar age- and race-adjusted association between serum tHcy concentrations and CHD prevalence in middle-aged women ($P=.04$) in a prospective case-cohort design, but again, no association was observed in men.

The efficacy of using folate, with or without vitamins B_6 and B_{12}, to reduce the prevalence of atherosclerotic vascular disease will be determined by randomized clinical trials, such as that recently reported by Vermeulen et al,[26] and the 7 trials reported by Clarke and Collins[27] that are being initiated or are underway with hard clinical end points, eg, stroke or myocardial infarction. However, even before the results of clinical trials become available, the role of folate as the principal determinant of high serum

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tHcy concentrations in the elderly could be changing. Following the completion of the NMEHS, and the Eikelboom study,[24] the US Food and Drug Administration published a regulation (1998) that all enriched grain products be fortified with folic acid.[28] The first report on the effect of folate fortification indicated a 92% reduction in the prevalence of folate concentrations <7 nmol/L, and a 48% reduction in the prevalence of serum tHcy concentrations >13 μmol/L,[29] an observation consistent with later reports.[30] An elevated serum tHcy does not necessarily signify a lack of folate, with alternative etiologies including renal insufficiency, vitamin B_{12} deficiency, poor pyridoxine (B_6) nutrition, and poor riboflavin nutrition. The relative importance of these etiologies can be expected to increase with improved folate status in the population. For clinicians evaluating elderly individuals with elevated tHcy, these etiologies should be investigated before initiation of folate supplementation, especially because of the concern that folate supplementation could "mask" the megaloblastic anemia of B_{12} deficiency. Considering the apparent improvements in population folate status due to fortification, it will be interesting to compare the results of our study of tHcy, vitamin status, and CHD prevalence with those of studies conducted after 1998.

ACKNOWLEDGMENTS

This work is supported by grants from the National Institute on Aging (R01AG10941) and UNM GCRC (NCRR-GCRC Grant M01RR0997).

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