SERUM TOTAL HOMOCYSTEINE LEVELS, FOLATE, AND B-VITAMINS INTAKE AND CORONARY HEART DISEASE RISK FACTORS AMONG TRI-ETHNIC COLLEGE STUDENTS

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The main objective of our study was to determine and compare total serum homocysteine (tHcy) levels among tri-ethnic college students. The 180 tri-ethnic subjects completed Cardiovascular Risk Assessment questionnaires, and gave 15 mL fasting blood for serum tHcy and blood lipid analysis. The mean tHcy (± SD) of the all subjects was 6.33 ± 3.15 μmol/L. Male subjects had significantly (P=.001) higher serum tHcy levels compared with female subjects. Black non-Hispanic females and Hispanic females showed significantly (P=.003) lower tHcy levels than White non-Hispanic females. Moderate elevations of tHcy levels were strongly related to cigarette smoking, physical inactivity, behavioral style, high blood pressure, and low intakes of folate, and vitamins B6 and B12. A positive association of tHcy levels with cardiovascular heart disease (CHD) risk point standards was observed in females (P=.001), Hispanic (P=.001), Hispanic males (P=.049), Hispanic females (P=.009), and Black non-Hispanic females (P=.005). We observed gender and ethnic differences in tHcy levels of this young population with normal tHcy levels. Abnormally high tHcy concentrations appear to be acquired later in life. (*Ethn Dis.* 2004;14:64–72)

Key Words: Homocysteine, Coronary Heart Disease Risk, Gender, Ethnicity, College Students, Folate, B-Vitamins

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INTRODUCTION

Coronary heart disease (CHD) is the single largest killer in the United States.[1] Conventional risk factors of CHD predict less than one half of the future cardiovascular events, and may not have the same effect among different ethnic groups.[2] A high concentration of an amino acid homocysteine (tHcy) in the blood is thought to enhance plaque formation and thus affect subsequent blockage of the arteries.[3–4] Hyperhomocysteinemia is now recognized as an independent risk factor for heart disease, including increased risk of occlusive vascular disease, peripheral vascular disease, and atherosclerosis.[5–12] Hyperhomocysteinemia has been reported to be as damaging as smoking or high blood cholesterol levels, causing myocardial infarction and coronary heart disease.[13–15] This association has been confirmed in several large studies involving adolescents and adults from different ethnic groups.[15–17] Also, associations between tHcy and conventional risk factors, such as cigarette smoking,[8,12,14] blood pressure,[8] and cholesterol have been demonstrated.[12,18] Hyperhomocysteinemia has been linked to inadequate intakes of folate, vitamins B6 and B12, and antioxidant vitamins. Supplementation with these vitamins has been shown to reduce blood tHcy levels.[3,19–28] Some patients may survive into adulthood despite prolonged exposure to elevated tHcy levels, but many develop typical generalized atherosclerosis.[29,30] Available information regarding tHcy levels among adolescents and young males and females from different ethnic background is limited.

The main objective of our study was to determine and compare serum tHcy levels among tri-ethnic college students (White non-Hispanic [WNH], Hispanic [H], and Black non-Hispanic [BNH]). The specific objectives were to: 1) Analyze the differences in distribution of serum tHcy; 2) Examine the relationship between serum tHcy and established cardiovascular risk factors computed as CHD risk point standards; and 3) Analyze the relationship between folate, B6, and B12 intake and serum tHcy by gender, ethnicity, and gender-ethnicity subgroups.

METHODS

Subject Recruitment and Selection

Three hundred college students at Florida International University (FIU) were recruited to participate in an American Heart Association (AHA) sponsored study. Subjects were recruited using flyers distributed in classrooms and areas where students socialize on campus. Subjects were college students at FIU during 1999–2000, whose ages were less than 40 years. Males and females, pursuing any field of study except nutrition were eligible as subjects. Since nutrition students possess greater knowledge of CHD prevention and treatment, they were excluded from the study to eliminate possible bias. Participants were required to originate from one of the targeted tri-ethnic groups. Students of Hispanic, Black non-Hispanic and White non-Hispanic origin were encouraged to report to the investigator's laboratory on campus to take part in the study. Only one visit, taking approximately 60 minutes to complete the entire process, was necessary to collect all of the required data. Subjects signed an informed consent form, approved by the FIU Institutional Review

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Board (IRB) prior to participation in the study. A total of 300 subjects were recruited to participate in the study. Since high power analysis was utilized, only 180 subjects out of 300 were analyzed for this study.

Data Collection

All subjects were required to complete the Cardiovascular Risk Assessment Instruments (CRAIs) containing a self-reported socio-demographic questionnaire. The Cardiac Risk Evaluation Questionnaire (CREQ) was used to measure CHD risk factors. Measured risk factors were quantified as "CHD risk point standards," which provided a quantitative interpretation of the risk factors of age, race, gender, diabetes, smoking, high blood pressure, high blood cholesterol, physical inactivity, obesity, and high stress level. The questionnaire also included the participant's family history of heart disease, personal history of heart disease, personal and family histories of diabetes, smoking habits, behavioral style, and activity levels. A fasting 15 ml blood sample was drawn into a vacutainer tube from the antecubital vein, using sterile, standard techniques, with the subject in the sitting position. After the blood in the Serum Separator Tube (SST) had been completely coagulated, a process requiring a minimum 30-minute waiting period, yet no later than a 45-minute delay after venipuncture, blood was centrifuged at full speed (1100 RCF) for 15 minutes. The serum was then transferred into 3 labeled plastic tubes. The first tube was used immediately for lipid analysis; the second was frozen and shipped for tHcy analysis; and the third was stored in the freezer at −70 C. for reserve.

Dietary Data Collection: Food Frequency Questionnaire (FFQ)

The FFQ was adapted from the Nurses' Health Study Dietary Questionnaire (Willett, 1990) and was intended to measure the usual, self-selected diets of individuals. Students were asked to recall how often they consumed food items, the method of preparation they used and the portion sizes they consumed compared to commonly-used standards. Dietary intakes of fat, saturated fat, cholesterol, and vitamins and minerals were determined from information included in the students' responses to the questionnaire. Current or past use of vitamin and/or mineral supplements (including vitamins A, C, E, B6, folic acid, B12, fish oil, beta carotene, iron, selenium, zinc, and calcium), or multiple vitamin/mineral preparations, dosage and brand name were included. The mean intake of vitamins was assessed from students' supplement intake and their diet. For each item on the questionnaire, respondents were given up to 9 choices ranging from never, or less than once per month, to 4 or more times per day. Information on the serving size and number of servings was also obtained. The FFQs were collected and coded by the investigators and analyzed by Channing Laboratories for individual nutrient intakes.

Analysis of Homocysteine Levels and Blood Lipids

Serum tHcy levels were measured using the IMx System, manufactured by Axis Biochemical ASA Ulvenveien 87, N-0581, Oslo, Norway, for Abbott Diagnostics Division. The IMx tHcy assay was based on the Fluorescence Polarization Immunoassay (FPIA) technology for the quantitative measurement of L-tHcy in human serum on the IMx Analyzer. Serum tHcy analysis were conducted at the Vascular Disease Intervention and Research Laboratory LLC, Edmond, Oklahoma. The serum total cholesterol (TC), high-density (HDL) and low-density lipoprotein (LDL) cholesterol, and triglyceride (TG) levels were measured using the BECKMAN Synchron CX System. Blood lipid analyses were done by Quest Diagnostics Laboratory, West Palm Beach, Florida.

Cardiovascular Risk Factors Analysis

A risk-point standard system based on 9 CHD risk factors and developed by the American Heart Association was used.[31] For each CHD risk factor, a zero or eight-risk level was assigned. The most significant risk factors for developing CHD were given the highest numeric point. Based on the combined results of the CHD Risk Evaluation Questionnaire (CREQ), the blood pressure results and the blood lipid levels, subjects were assigned a score ranging from zero to 8 (the maximum number of points for each factor). The risk points from all of the risk factors were tallied; the risk category was determined using the following category list:

Risk Point Standards

Risk category	Points
Well-below average (low CHD risk)	5–15
Below average, good	16–23
Borderline, fair	24–35
Above average, poor	36–44
Very high, very poor	45–60
Dangerously high (high CHD risk)	Above 60

Statistical Analysis

Data from the CREQ, FFQ, and tHcy analysis were analyzed by gender and ethnicity using a SPSS Computer Program. The results were expressed as mean ± standard deviation, or frequen-

cies and percentages. One way Analysis of Variance (ANOVA) was used to determine the mean differences in CHD risk point standards and serum tHcy among tri-ethnic students by gender, ethnicity, and gender-ethnicity subgroup. Pearson's correlation was used to determine the association of various independent variables (IVs) to "CHD risk point standards." Differences were considered significant at *P* value less than .05.

RESULTS

One hundred eighty subjects, 50% male and 50% female equally representing the tri-ethnic groups participated in the study. Serum tHcy levels were determined for 177 subjects. Of this number, 49.2% (*N*=87) were males, and 50.8% (*N*=90) were females. One-third of participants (*N*=59) were White non-Hipanic, Hispanic and Black non-Hispanic. In each of the male-ethnicity subgroups, 29 subjects were represented. The number increased by 1 to 30 subjects in each of the female-ethnicity subgroups (Table 1).

Table 1. Mean serum total homocysteine concentrations by gender, ethnicity, and gender-ethnicity subgroup

Variable	*N* (%)	Mean ± SD	F	*P* value
All subjects	177 (100)	6.33 ± 3.15		
Gender				
Male	87 (49.2)	7.18 ± 3.80[b]		
Female	90 (50.8)	5.51 ± 2.09[a]	13.40	.001*
Ethnicity				
White non-Hispanic	59 (33.3)	6.70 ± 3.60		
Hispanic	59 (33.3)	6.27 ± 2.20		
Black non-Hispanic	59 (33.3)	6.02 ± 3.51	0.681	.508
Male, by ethnicity				
Male White non-Hispanic	29 (16.4)	6.86 ± 4.70		
Male Hispanic	29 (16.4)	7.43 ± 2.00		
Male Black non-Hispanic	29 (16.4)	7.27 ± 4.25	0.171	.843
Female, by ethnicity				
Female White non-Hispanic	30 (16.9)	6.54 ± 2.10[b]		
Female Hispanic	30 (16.9)	5.16 ± 1.80[a]		
Female Black non-Hispanic	30 (16.9)	4.82 ± 2.03[a]	6.35	.003†

* $P<.001$.
† $P<.01$.
[a,b] Means in a column with different superscripts are statistically significant using Bonferroni's procedure at $P<.05$.

Homocysteine

The mean (± SD) tHcy level for all subjects was 6.33 ± 3.15 μmol/L. Males showed significantly (*P*=.001) higher mean tHcy concentration (7.18 ± 3.80) than females (5.51 ± 2.09 μmol/L). Black non-Hispanic (BHN) females showed a significantly (*P*=.003) lower (4.82 ± 2.03 μmol/L) tHcy concentration than WNH females (6.54 ± 2.10 μmol/L), and H females showed a significantly lower (5.16 ± 1.80) tHcy concentration than WNH females (6.54 ± 2.10 μmol/L) (Table 1).

Correlations between Homocysteine Levels and Lifestyle Factors

A significant and positive correlation was found between tHcy levels and cigarette smoking in females (*r*=0.336, *P*=.001), H (*r*=0.337, *P*=.009), BNH (*r*=0.332, *P*=.010), H males (*r*=0.386, *P*=.039), and in BNH females (*r*=0.547, *P*=.002). Significant inverse correlations were found between tHcy levels and the amount of exercise in H males (*r*=−0.500, *P*=.006), and in WNH females (*r*=−0.433, *P*=.014). Significant positive correlations were found between tHcy levels and behavioral style in females (*r*=0.243, *P*=0.021), in H (*r*=0.260, *P*=0.046), and in H females (*r*=0.508, *P*=.004). A significant positive correlation was found between tHcy levels and systolic blood pressure in females (*r*=0.221, *P*=.036), H (*r*=0.325, *P*=.012), and in BNH (*r*=0.258, *P*=.048) (Table 2).

CHD Risk Point Standards

A significant positive correlation was found between tHcy levels and "CHD risk point standards" in females (*r*=0.336, *P*=.001), in H (*r*=0.426, *P*=.001), in H males (*r*=0.368, *P*=.049), H females (*r*=0.471, *P*=0.009), and BNH females (*r*=0.496, *P*=.005) (Table 3).

Dietary Intake

The mean intakes of protein (gm) and methionine (gm) were 100.3 ± 53.9 and 2.4 ± 1.3, respectively. For men, the dietary analysis reflected significantly (*P*=.001) higher protein (114.1 ± 62.5 vs 86.5 ± 39.4) and methionine (2.7 ± 1.6 vs 2.1 ± 0.1) intake than females. White non-Hispanic (WNH) subjects showed a significantly (*P*=.028) higher intake of protein and methionine (*P*=.039) than BNH subjects (111.4 ± 65.6 vs 91.5 ± 42.1 and 2.7 ± 1.6 vs 2.2 ± 1.0, respectively). White non-Hispanic (WNH) men had significantly (*P*=.003) higher protein and methionine (*P*=.005) intake than BNH males (138 ± 75.6 vs 99.7 ± 48.9 and 3.3 ± 1.9 vs 2.4 ± 1.2, respectively). Also, WNH males had significantly (*P*=.003) higher intake of protein and methionine (*P*=.005) than H males (138 ± 75.6 vs 104.5 ± 53.6 and 3.3 ± 1.9 vs 2.5 ± 1.4, respectively) (Table 4).

Table 2. Correlations between homocysteine levels and lifestyle factors by gender, ethnicity, and gender-ethnicity subgroup

Variable	Smoking	Physical Activity	Behavioral Style SBP	r–*P* value
Gender				
Male	0.094–**0.398**	NS	−0.027–**0.801**	0.072–**.510**
Female	0.336–**0.001***	NS	0.243–**0.021**‡	0.221–**.036**‡
Ethnicity				
White non-Hispanic	0.018–**0.895**	NS	0.068–**0.607**	0.095–**.476**
Hispanic	0.337–**0.009**†	NS	0.260–**0.046**‡	0.325–**.012**‡
Black non-Hispanic	0.332–**0.010**†	NS	0.184–**0.163**	0.258–**.048**‡
Male, by ethnicity				
Male White non-Hispanic	0.057–**0.770**	−0.058–**0.765**	NS	NS
Male Hispanic	0.386–**0.039**‡	−0.500–**0.006***	NS	NS
Male Black non-Hispanic	0.157–**0.417**	0.189–**0.327**	NS	NS
Female, by ethnicity				
Female White non-Hispanic	0.210–**0.267**	−0.433–**0.014**‡	0.167–**0.377**	NS
Female Hispanic	0.162–**0.392**	0.055–**0.772**	0.508–**0.004**†	NS
Female Black non-Hispanic	0.547–**0.002**†	−0.209–**0.267**	0.037–**0.848**	NS

* *P*<.001.
† *P*<.01.
‡ *P*<.05.
NS=none significant; SBP=systolic blood pressure.

Vitamins

Vitamin Intake with Supplements

The mean daily intakes of vitamins B6 (5.6 ± 19.0 mg), B12 (10.0 ± 10.9 ìg), and folate (439.5 ± 277.7 ìg) exceeded the Dietary Reference Intakes (DRIs). Hispanic (H) males had significantly lower mean vitamin B12 (*P*=.046) (9.1 ± 5.7) and folate intakes (*P*=.032) (409.5 ± 195.6) than WNH males (12.6 ± 9.1 and 551.4 ± 396.2, respectively). Black non-Hispanic (BNH) males also had significantly (*P*=.032) lower mean folate intakes (420.2 ± 264.6) than WNH males (551.4 ± 396.2) (Table 5).

Vitamin Intake without Supplements

Mean vitamin intakes excluding supplements, exceeded the DRIs for vitamin B6 (2.3 ± 1.2), and vitamin B12 (7.6 ± 7.0), whereas intakes of folate (350.0 ± 183.9) did not meet the dietary guidelines. Without supplementation, WNH males consumed significantly higher amounts of vitamin B6 (*P*=.003) (3.0 ± 1.6) than H males (2.3 ± 1.0) and BNH males (2.3 ± 1.0). Also, WNH males consumed significantly higher levels of folate (*P*=.016) (450.8 ± 284.1) than H males (342.0 ± 131.9) and BNH males (350.4 ± 177.6). Among females overall, H females consumed significantly higher mean intakes of vitamin B12 than WNH and BNH females (*P*=.018) (8.34 ± 9.90 vs 5.12 ± 4.50 for WNH, and 5.03 ± 3.17 for BNH) (Table 5).

A significant inverse correlation was found between tHcy levels, vitamin B12

Table 3. Correlation between homocysteine levels and CHD risk point standards by gender, ethnicity, and gender-ethnicity subgroup

Variable	CHD Risk Point Standards r–*P* value
Gender	
Male	−0.013–**.907**
Female	0.336–**.001***
Ethnicity	
White non-Hispanic	−0.056–**.676**
Hispanic	0.426–**.001***
Black non-Hispanic	0.149–**.259**
Male, by ethnicity	
Male White non-Hispanic	−0.190–**.323**
Male Hispanic	0.368–**.049**‡
Male Black non-Hispanic	−0.207–**.280**
Female, by ethnicity	
Female White non-Hispanic	0.233–**.215**
Female Hispanic	0.471–**.009**†
Female Black non-Hispanic	0.496–**.005**†

* *P*<.001.
† *P*<.01.
‡ *P*<.05.

Table 4. Mean intakes of total protein and methionine by gender, ethnicity, and gender-ethnicity subgroup

Variables	Total Protein (gm)	Methionine (gm)
	Mean ± SD	
All subjects	100.3 ± 53.9	2.4 ± 1.3
Gender		
Male	114.1 ± 62.5	2.7 ± 1.6
Female	86.5 ± 39.4	2.1 ± 0.1
P value	**.001***	**.001***
Ethnicity		
White non-Hispanic	111.4 ± 65.6[b]	2.7 ± 1.6[b]
Hispanic	98.0 ± 50.0[ab]	2.4 ± 1.3[ab]
Black non-Hispanic	91.5 ± 42.0[a]	2.2 ± 1.0[a]
P value	**.028‡**	**.039‡**
Male, by ethnicity		
Male White non-Hispanic	138.0 ± 75.6[b]	3.3 ± 1.9[b]
Male Hispanic	104.5 ± 53.6[a]	2.5 ± 1.4[a]
Male Black non-Hispanic	99.7 ± 48.9[a]	2.4 ± 1.2[a]
P value	**.003†**	**.005‡**
Female, by ethnicity		
Female White non-Hispanic	85.0 ± 39.1	2.1 ± 0.1
Female Hispanic	91.6 ± 45.9	2.2 ± 1.1
Female Black non-Hispanic	83.2 ± 32.4	2.0 ± 0.8
P value	**.537**	**.546**

* $P<.001$.
† $P<.01$.
‡ $P<.05$.
[a,b] Means in a column with different superscripts are statistically significant using Bonferroni's procedure at $P<.05$.

($r=-0.169$, $P=.025$), and folate intakes ($r=-0.191$, $P=.011$). In males ($r=-219$, $P=.042$) and females ($r=-0.236$, $P=.025$), a significant inverse correlation was found between tHcy levels and folate intake. In females, significant inverse correlations were found between tHcy levels and vitamins B6 ($r=-0.282$, $P=.007$), and vitamin B12 ($r=-0.323$, $P=.002$). In BNHs, a significant inverse correlation was found between tHcy levels and vitamin B12 ($r=-0.280$, $P=.031$). Significant inverse correlations were found between tHcy levels and vitamin B6 ($r=-0.402$, $P=.028$) and Vitamin B12 intakes ($r=-0.339$, $P=.029$) in BNH females. A significant inverse correlation was only found between serum tHcy and vitamin B12 intakes without supplement ($r=-0.374$, $P=.046$) in H males (Table 6).

DISCUSSION

Gender and Ethnic Differences

This study presented baseline data gathered from a tri-ethnic college-age population to determine serum tHcy levels. The results revealed that tHcy concentrations increase with age, especially among WNH females. Mean tHcy concentrations were observed to be higher in males than in females in all gender-ethnic subgroups. The age and gender differences found in our study are consistent with observations from other studies of similar age and gender groups.[12,16,25,27] Significant age-gender interaction revealed that tHcy concentrations in females tend to diverge from

Table 5. Mean intake vitamins with and without supplements by gender-ethnicity subgroup

Vitamins	B-6 mg	B-12 µg	Folate µg	B-6 mg	B-12 µg	Folate µg
	Mean ± SD with Supplement			Mean ± SD without Supplement		
All subjects	5.63 ± 19.0	10.0 ± 10.9	439.5 ± 277.7	2.3 ± 1.2	7.6 ± 7.0	350.0 ± 183.9
Male, by ethnicity						
Male White non-Hispanic	4.13 ± 3.6	12.6 ± 9.1[b]	551.4 ± 396.2[b]	3.0 ± 1.6[b]	10.7 ± 8.1	450.8 ± 284.1[b]
Male Hispanic	3.15 ± 4.2	9.1 ± 5.7[a]	409.5 ± 195.6[a]	2.3 ± 1.0[a]	8.1 ± 5.5	342.0 ± 131.9[a]
Male Black non-Hispanic	10.70 ± 41.1	9.4 ± 7.7[ab]	420.2 ± 264.6[a]	2.3 ± 1.0[a]	8.5 ± 7.0	350.4 ± 177.6[a]
P value	**.237**	**.046‡**	**.032‡**	**.003†**	**.144**	**.016‡**
Female, by ethnicity						
Female White non-Hispanic	4.41 ± 8.5	7.6 ± 8.3	421.5 ± 260.6	1.9 ± 0.9	5.1 ± 4.5[a]	314.4 ± 127.8
Female Hispanic	3.15 ± 3.9	9.3 ± 10.2	387.5 ± 216.5	2.2 ± 1.0	8.3 ± 9.9[b]	322.0 ± 157.5
Female Black non-Hispanic	8.28 ± 18.9	11.8 ± 19.0	447.2 ± 273.4	2.0 ± 0.8	5.0 ± 3.2[a]	320.3 ± 150.9
P value	**.094**	**.299**	**.494**	**.300**	**.018‡**	**.963**

† $P<.01$.
‡ $P<.05$.
[a,b] Means in a column with different superscripts are statistically significant using Bonferroni's procedure at $P<.05$.

Table 6. Correlations between homocysteine levels and B-vitamins by gender, ethnicity, and gender-ethnicity subgroup

Vitamins	B-6 mg	B-12 μg	Folate μg	B-6 mg	B-12 μg	Folate μg
	r, With Supplement			r, Without Supplement		
All subjects	−0.064	−0.169‡	−0.191‡	−0.036	−0.017	−0.102
Gender						
Male	−0.027	−0.130	−0.219†	−0.089	−0.074	−0.153
Female	−0.282†	−0.323†	−0.236‡	−0.118	−0.150	−0.154
Ethnicity						
White non-Hispanic	−0.112	−0.102	−0.208	−0.065	−0.018	−0.125
Hispanic	−0.129	−0.027	−0.163	−0.100	−0.086	−0.021
Black non-Hispanic	−0.070	−0.280*	−0.230	−0.165	−0.115	−0.149
Male, by ethnicity						
Male White non-Hispanic	−0.096	−0.107	−0.184	−0.034	−0.061	−0.133
Male Hispanic	−0.155	−0.232	−0.226	−0.029	−0.374‡	−0.069
Male Black non-Hispanic	−0.039	−0.291	−0.283	−0.291	−0.254	−0.284
Female, by ethnicity						
Female White non-Hispanic	−0.211	−0.246	−0.351	−0.122	−0.056	−0.245
Female Hispanic	−0.047	−0.191	−0.136	−0.152	−0.177	−0.042
Female Black non-Hispanic	−0.402‡	−0.399‡	−0.256	−0.233	−0.192	−0.144

† $P<.01$.
‡ $P<.05$.

those in males at younger ages and converge at older ages. The Hordaland Study found that tHcy levels were higher in males (10.1 μmol/L) than in females (9.0 μmol/L).[12] The Framingham Study,[27] also reported the tHcy concentrations as higher in males (11.8 μmol/L) than in females (10.7μmol/L) aged 75 years or younger. The explanation for the differences among genders is not clear. However, many believe that the explanation may be related to folate, vitamins B6 and B12 intakes,[27,32] muscle mass and hormonal factors.[33] Lower tHcy levels among young females may explain, in part, the lower rates of CHD among them before the age of menopause.

Few studies have looked at the role ethnic differences may play in tHcy levels.[16,17] We found that BNH and H females had significantly lower tHcy than WNH females. Jacques et al found higher mean tHcy concentration in WNH females than in Mexican-American females.[16] Our findings are consistent with their data. Across ethnic groups, we did not find significant differences in tHcy in males. However, in another study, South African Black males were found to have lower tHcy concentrations than South African White males.[17] Nevertheless, a later comparison of college-age subjects, living under similar conditions, did not reflect these differences in tHcy concentrations. After methionine load tests, Blacks had lower tHcy levels than Whites, a finding which may explain their relative resistance to CHD. Furthermore, our findings indicated that BNH females had lower tHcy concentrations as compared to WNH and H females.

tHcy and Conventional Cardiovascular Disease (CVD) Risk Factors

Our findings identified a strong association between cigarette smoking and tHcy concentration, especially in females. This finding extended to different ethnic groups, including BNH and H; and, within the gender-ethnic subgroups, this correlation was also discovered to be strong. With the number of cigarettes smoked per day, tHcy increases proportionally according to our research; our findings correspond with other studies[12,14] and contrasted in another.[33] In the Hordaland Study, a dose-response relationship was found between cigarette smoking and tHcy, especially in females of older age groups.[12] Overall, the increase in tHcy level per cigarettes smoked per day was 1% in females and 0.6% in males. The cause of increased tHcy levels among smokers is not known. tHcy levels may conceivably augment smoking-related platelet and clotting effects exerting a toxic effect on the endothelium, thereby effecting the pathogenesis of vascular disease. Lower intake of vitamins, such as folate and vitamins B6 and B12, have also been reported in smokers.[34–36]

In our study, serum tHcy levels were inversely related to physical activity, especially in females. This inverse relationship was strong within the gender-ethnic groups, such as in H males and WNH females. Vigorous and heavy physical activity conferred a further reduction in tHcy levels, according to our research. suggesting that subjects with hyperhomocysteinemia may benefit from exercise in lowering their tHcy lev-

el in addition to changing other lifestyle factors such as smoking. A similar relationship has been suggested in the Hordaland Study, where they demonstrated for the first time that the tHcy level was inversely related to physical activity.[12] And one study has demonstrated a dose-response reduction in CHD risk with physical activity.[37]

Our results showed that serum tHcy levels are positively related to stress level, especially in females. Acute and chronic stress may influence other CHD risk factors, such as smoking, high-cholesterol level, physical inactivity, and obesity. Other studies have examined the relationship between tHcy and stress.[38–40] Researchers found significant elevations in tHcy levels during acute psychological stress, with return to baseline levels during recovery.[38] Yet another study reported that stress management can decrease tHcy concentration.[39]

Serum tHcy level was positively related to systolic blood pressure (SBP), especially in females, according to our results. This relationship was strong among H and BNH female subjects. No association was found with diastolic blood pressure (DBP)—a relationship that has been documented in other studies.[7,10,12,38,41] In the Hordaland Study, tHcy levels correlated with SBP and DBP among the younger subjects. In the child and adolescent trial for cardiovascular health, the serum tHcy level was only related to SBP; and after adjustment for other risk factors, tHcy remained independently associated with SBP.[41]

Vitamin Intakes

Our results showed that high serum tHcy levels are inversely related to low intakes of folic acid, vitamins B6 and B12. This relationship was strong among females, especially for BNH females who had higher intakes when supplements included folate, vitamins B6, and B12. Their mean tHcy levels were significantly lower than WNH females. Without supplements, lower intakes of folate (below the DRIs) and vitamin B6 were observed in H and BNH males compared to WNH males. Our data showed that college students met their daily recommended dietary requirements for vitamins when they used supplements. This finding is supported by others, who have indicated that the use of fortified foods and vitamin supplements are very common in the United States. More than 50% of college students use a high level of supplements; a possible outcome may be a reduction in their total tHcy levels.[42]

High levels of vitamin intakes, especially folate and vitamins B6 and B12, are correlated with lower tHcy levels in the US population.[15] Since these vitamins are cofactors in the conversion of tHcy to methionine and cysteine, hyperhomocysteinemia is considered a stronger risk factor for CHD among younger subjects than older subjects.[4] The Nurses' Health Study documented that dietary intakes of folate and vitamin B6, but not vitamin B12, were insufficient among 80,000 females.[23] This study also indicated an inverse relationship between dietary intake of these vitamins and mortality and morbidity from cardiovascular disease during a 14-year period. The Norwegian Study and the Framingham Heart Study demonstrated that deficiencies of folate, vitamins B6 and B12 intakes are associated with elevated tHcy levels.[3,27] The National Academy of Sciences, Institute of Medicine recommends folate intake of 400 μg/day. The NHANES III data indicate that the average dietary folate intake of US adults is 283 μg/day.[43] Furthermore, the NHANES III data demonstrated that age-specific mean folate and vitamin B12 intakes were generally higher in WNHs followed by Mexican Americans, and lowest in BNH males and females.[16] Researchers have theorized that among children with homocystinuria, atherogenesis is secondary to hyperhomocysteinemia caused by dietary deficiencies of folate, vitamins B6 and B12, and toxic factors such as smoking.[6,7,10–11] This theory also supports our hypothesis that high folate and vitamins B6 and B12 intakes would be associated with lower tHcy levels, therefore, with lower risk for CHD.

Healthy eating habits, including adequate intake of vitamins, especially folate, B6 and B12, may offer protection against CHD.

CONCLUSION

In the present study, moderate elevation of tHcy levels were strongly related to male gender, cigarette smoking, physical inactivity, behavioral style, high blood pressure, and low-folate and vitamin B6 and B12 intakes. Black non-Hispanic and Hispanics had lower tHcy levels compared to White non-Hispanics. The low tHcy concentration among the Black population may partially explain the low prevalence of CHD in this ethnic group. Healthy eating habits, including adequate intake of vitamins, especially folate, B6 and B12, may offer protection against CHD.

Based on our findings and other research, and given that tHcy concentrations are low in WNH, H, and BNH college-age individuals, it may be concluded that abnormally high tHcy concentrations may be acquired later in life. Future longitudinal studies are needed to establish whether gender-ethnic differences in tHcy metabolism may offer protection against CHD.

Limitations

The study population was formed among a modest number of college students, who were conveniently selected for this sample. The results may not be generalized to all college-age individuals

or to other populations. The findings of this study will require confirmation in a population of randomly selected young adults outside of a university setting.

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