Genetic, dietary, and other lifestyle determinants of serum homocysteine levels in young adults in Costa Rica

Ileana Holst-Schumacher,[1] Rafael Monge-Rojas,[2] Priscilla Cambronero-Gutiérrez,[1] and Gilbert Brenes[3]

ABSTRACT

Objective.   *Elevated serum total homocysteine (tHcy) is considered an independent risk factor for cardiovascular disease. The objective of this study was to develop the first-ever information on the prevalence of hyperhomocysteinemia and its determinants in a population in Costa Rica.*

Methods.   *A cross-sectional study was conducted to determine serum levels of tHcy, vitamin B_{12}, folate, and creatinine, as well as the presence of the genotype TT for the methylenetetrahydrofolate reductase (MTHFR) enzyme. Additionally, dietary vitamin intakes and other lifestyle risk factors were assessed. A total of 399 Costa Rican adults from the central valley of the country (where the capital city, San José, is located), aged 20 to 40 years, participated in this study in the year 2000. Analyses of variance were performed for continuous variables, and the chi-square test was used for categorical data. Spearman correlation tests were calculated to determine associations between variables. Three linear regression analyses and one binary logistic model were developed in order to determine the predictors for homocysteine levels in the population studied.*

Results.   *The overall prevalence of hyperhomocysteinemia (> 15 µmol/L) in the population was 6%, 31% of the population were in the range of 10 to 15 µmol/L, 29% had the genotype TT for the enzyme MTHFR, 18% presented a vitamin B_{12} deficiency (< 165 pmol/L), and none of the persons had low serum folate levels (< 7.0 nmol/L). No significant associations were found between tHcy and age, smoking, consuming alcohol, or dietary vitamin intake.*

Conclusions.   *Only serum vitamin B_{12} levels and the genotype TT of the enzyme MTHFR were considered significant predictors of high serum tHcy levels in the Costa Rica population studied.*

Key words   Cardiovascular diseases, homocysteine, life style, risk factors, Costa Rica.

[1] University of Costa Rica, Investigation Center for Abnormal Hemoglobins and Related Sicknesses, San Pedro de Montes de Oca, San José, Costa Rica. Send correspondence and requests for reprints to: Ileana Holst-Schumacher, Investigation Center for Abnormal Hemoglobins and Related Sicknesses (CIHATA), University of Costa Rica, Postal Code 2060, San Pedro de Montes de Oca, San José, Costa Rica; fax: (506) 225-2374/(506) 207-5440; telephone: (506) 207-5440/(506) 207-4389; e-mail: iholst@cariari.ucr.ac.cr

[2] Costa Rican Institute for Research and Education on Nutrition and Health, Tres Ríos, Cartago, Costa Rica.

[3] University of Costa Rica, Central American Population Center and School of Statistics, San Pedro de Montes de Oca, San José, Costa Rica.

Since 1970, researchers have linked hyperhomocysteinemia to cardiovascular disease (CVD), and hyperhomocysteinemia is now considered a CVD risk factor (1). Low serum concentrations of folate, vitamin B_{12}, vitamin B_6, and vitamin B_2 have been identified as risk factors for hyperhomocysteinemia (2). Folate is considered the micronutrient with the greatest impact on homocysteine metabolism (2).

Several genetic defects in the enzymes participating in the homocysteine metabolic cycle have also been associated with hyperhomocysteinemia (3). Frosst et al. (4) identified a common point mutation (C677T) in the methylenetetrahydrofolate reductase (MTHFR) gene that correlated with reduced MTHFR activity. Homozygotes for this enzyme variant have significantly elevated total homocysteine (tHcy) levels, suggesting that this mutant gene may be a risk factor for developing hyperhomocysteinemia (3, 4).

Moderate hyperhomocysteinemia is equivalent to hypercholesterolemia as a cardiovascular risk factor. Given that, some researchers have pointed to the importance of knowing and reducing the tHcy concentrations in a population in order to lower CVD mortality (5). This is important in Costa Rica, where CVDs constitute the leading cause of death among adults, with a mortality rate of 80.4/10 000 in 2001, and where approximately 2.5% of those deaths occurred among relatively young persons, that is, under 40 years of age (6).

Some researchers have studied the traditional CVD risk factors among adults in Costa Rica (7), but there are no data available on the prevalence of hyperhomocysteinemia and its determinants in the country. This is the first study that describes the distribution of tHcy and its determinants among Costa Rican adults.

MATERIALS AND METHODS

Subject selection

A sample of 399 Costa Rican volunteers aged 20 to 40 years participated in this study, which was conducted in the year 2000. Subjects were asked to participate in this study through a written circular sent home with 700 randomly selected boys and girls from 20 representative rural and urban schools located in the central valley of the country, where the capital of the country, San José, is located. A total of 490 adults offered to collaborate with the investigation, but only 399 of them fulfilled all the inclusion and exclusion criteria.

The final sample consisted of 186 men and 213 women. Written informed consent was obtained from all the participants, and all the procedures that were followed had been approved in accordance with the guidelines of the bioethics committees of the University of Costa Rica and the Costa Rican Institute for Research and Education on Nutrition and Health. Inclusion criteria included being of Costa Rican nationality and being between 20 and 40 years old. The exclusion criteria included a serum creatinine concentration > 133 µmol/L and conditions such as thyroid disease, cancer, pregnancy, and exposure to nitrous oxide. Also excluded were people taking vitamin supplements, anticonvulsants, or antidepressant drugs, as well as women using estrogen therapy.

Study variables

The study data were collected through validated instruments in face-to-face interviews. The information included age, gender, and the consumption of vitamin supplements or any doctor-prescribed drug. (We excluded from this study all persons who said that they were taking vitamin supplements.) We also registered the presence or absence of cigarette smoking, coffee consumption, and alcoholic-beverage consumption.

Dietary intake was determined by semiquantitative 24-hour recall. Portion size was estimated using a series of photographs of food typically consumed in Costa Rica, along with three-dimensional food models. The Food Processor nutrition analysis software, version 6.0 for Windows (Esha Research, Salem, Oregon, United States), was used to perform nutrient calculations from the dietary data (8). A comparison with the United States Dietary Reference Intakes (DRIs) for folate, vitamin B_6, and vitamin B_{12} was made to evaluate micronutrient intake. Two-thirds or less of a DRI was used as the criterion for inadequate dietary intake of a micronutrient (9).

Blood measurements included tHcy, folate, vitamin B_{12}, and creatinine. After 8 to 12 hours of fasting, a venous blood sample was collected from each subject, following National Committee for Clinical Laboratory Standards procedures (10). A plain Vacutainer tube (Becton, Dickinson and Company, Rutherford, New Jersey, United States) was used to obtain serum, and another tube with ethylenediaminetetraacetate (EDTA) was used for DNA extraction. Serum was separated immediately from the blood cells and refrigerated (6 °C ± 2 °C) until further processing. Ascorbic acid (5 000 mg/L) was added to the serum aliquot that was reserved for folate analysis in order to preserve the reduced state and stability of the vitamin, as suggested by Henry et al. (11). Samples were subsequently stored at –20 °C until analyzed.

Biochemical and genetic analysis

The serum tHcy level was measured using fluorescence polarization immunoassay, and serum folate and vitamin B_{12} levels were determined with microparticle immunoassays in a fully automated IMx (Abbott Laboratories, Diagnostics Division, Abbott Park, Illinois, United States) (12). Total homocysteine serum levels were categorized as normal (< 10 µmol/L), risky (10 to 15 µmol/L), or high (> 15 µmol/L) (12, 13). Creatinine was analyzed by kinetic reactions in a dry chemistry automated VITROS 250 (Johnson & Johnson, Ortho-Clinical Diagnostics, Rochester, New York, United States) (14). The assays' intra-assay coefficients of variation were 8% for tHcy, 12% for folate, < 6% for vitamin B_{12},

TABLE 1. Selected characteristics of the population in study of determinants of serum homocysteine levels, Costa Rica, 2000

Variable	Men (n = 186)	Women (n = 213)	Total (n = 399)
Age (y) (mean ± SD) (95% CI)[a,b]	32.9 ± 5.5 (32.2–33.7)	32.2 ± 5.7 (31.4–32.9)	32.5 ± 5.6 (32.0–33.1)
Folate (nmol/L) (mean ± SD) (95% CI)	25.4 ± 4.8 (24.8–26.1)	27.6 ± 3.9 (27.1–28.1)	26.7 ± 4.5 (26.2–27.0)
Vitamin B_{12} (pmol/L) (mean ± SD) (95% CI)	267 ± 122.0 (250.0–286.0)	268 ± 129.0 (251.0–286.0)	268 ± 125.0 (256.0–281.0)
Total homocysteine (µmol/L) (mean ± SD) (95% CI)	10.81 ± 3.0 (10.38–11.25)	8.26 ± 2.3 (7.94–8.57)	9.45 ± 2.9 (9.16–9.74)
Creatinine (µmol/L) (mean ± SD) (95% CI)	88.4 ± 8.8 (82.8–96.2)	70.7 ± 8.8 (65.2–87.7)	79.6 ± 8.8 (73.5–86.2)
Cigarette smoking			
Nonsmoker (%)	73.3	90.1	82.3
Smoker (%)	26.7	9.9	17.7
Coffee consumption			
Nondrinker or occasional drinker(%)[c]	24.1	23.0	23.5
Drinker (%)	75.9	77.0	76.5
Alcohol consumption			
Nondrinker (%)[d]	47.1	74.2	61.5
Drinker (%)	52.9	25.8	38.5

[a] SD = standard deviation.
[b] 95% CI = 95% confidence interval.
[c] Coffee nondrinker or occasional drinker = does not drink coffee or drinks < 1 cup per month.
[d] Alcohol nondrinker = drinks < 1 drink/month.

and < 3% for creatinine. Genomic DNA was isolated from blood leukocytes using the method of Miller et al. (15). Identification of the C to T substitution at nucleotide 677 of the MTHFR gene was assayed using the method of Frosst et al. (4). Thirty-five cycles at 95 °C for 60 s, 55 °C for 60 s, and 72 °C for 90 s were used to amplify 198-base pair (bp) products. The restriction enzyme *Hin*f I (Promega Corporation, Madison, Wisconsin, United States) digested the 198-bp fragment into 175-bp and 23-bp fragments, and the analysis was conducted in 3% agarose (Sigma-Aldrich Corporation, St. Louis, Missouri, United States) electrophoresis. Genetic analyses were performed for only a subset of the population studied (215 of 399) because of the high costs of these techniques. The genetic analyses were done according to the tHcy levels of those persons (102 with tHcy < 9.80 µmol/L, and 113 with tHcy > 10.20 µmol/L).

Data analysis

Statistical analysis was performed using SPSS software, version 9.1 for Windows (SPSS Inc., Chicago, Illinois, United States). Continuous variables are summarized using means ± standard deviation, and categorical variables are presented as frequency distributions. Analysis of variance was performed for continuous variables, and chi-square tests were performed for categorical data. Spearman correlation coefficients were calculated to determine associations between variables. Mann-Whitney nonparametric tests were computed to compare mean levels of serum biochemical indicators among homozygous normal (CC) genotype and heterozygous (CT) genotype, and between TT and non-TT subjects, since the distribution of these variables was skewed. Significance was defined as $P < 0.05$. The serum tHcy concentration (the dependent variable) was positively skewed, so the analyses were performed using natural logarithmic transformations. Inverse transformations were done to provide geometric means of tHcy, with 95% confidence intervals, adjusted by age and gender. Continuous variables were also categorized, using their quartiles as cutoff points. Three multiple linear regression models were developed to determine the best predictors of homocysteine levels. Additionally, a binary logistic model was developed, using serum tHcy concentration < 10 µmol/L or ≥ 10 µmol/L as the dependent variable.

RESULTS

General characteristics of the population

The study sample consisted of 399 adults (186 men and 213 women), with a mean age of 32.5 y (± 5.6 y) (range 20 to 40 y) (Table 1). A smoking habit was reported by 18% of the adults (27% of the men vs. 10% of the women, $P < 0.001$), and 38% reported consuming alcoholic beverages. In contrast, 77% of the participants were coffee drinkers.

Biochemical parameters of the population

Women showed higher mean serum folate levels than men did (27.6 and 25.4 nmol/L, respectively; $P < 0.001$), but men had higher mean serum levels of tHcy and creatinine than did women ($P < 0.001$) (Table 1). No significant differences were found by gender in the mean serum vitamin B_{12} concentrations.

The prevalence of hyperhomocysteinemia (> 15 µmol/L) in the total population was 6% (22 of 399), with the prevalence in men (19 of 186, or 10%), being much higher than that in women (3 of 213, or 1%) ($P < 0.001$). In terms of ranges, 31% of the adults had

TABLE 2. Distribution (%) of the population in study of determinants of serum total homocysteine concentrations, according to the total homocysteine concentration categories and cutoff values of serum folate and vitamin B_{12}, Costa Rica, 2000

Total homocysteine	Folate (nmol/L)		Vitamin B_{12} (pmol/L)		P value[a]
	< 7.0 (n = 0)	≥ 7.0 (n = 399)	< 165 (n = 70)	≥ 165 (n = 329)	
< 10 µmol/L	0.0	63.9	42.8	69.9	0.001
10–15 µmol/L	0.0	30.6	48.6	27.1	0.001
> 15 µmol/L	0.0	5.5	8.6	3.0	0.066
Total	0.0	100.0	100.0	100.0	

[a] P value according to chi-square test.

tHcy concentrations ranging between 10 and 15 µmol/L (50% of the men vs. 14% of the women, $P < 0.001$), and 64% showed normal tHcy levels (< 10 µmol/L) (85% of the women vs. 40% of the men, $P < 0.001$). The prevalence of hyperhomocysteinemia was 5% among those 20–29 years old, and 6% among those 30–40 years old.

None of the adults in this study presented low serum concentrations of folate (< 7.0 nmol/L) (Table 2). In contrast, 70 of the 399 adults (18%) showed deficient levels of serum vitamin B_{12} (< 165 pmol/L), 34 of 70 (49%) of the adults with low serum levels of vitamin B_{12} showed risky serum levels of tHcy (10–15 µmol/L, $P < 0.001$), and 6 of 70 (9%) had hyperhomocysteinemia. Serum creatinine showed a strong direct correlation with tHcy ($r = 0.472$), while folate and vitamin B_{12} presented an inverse relationship with it ($r = -0.267$ and $r = -0.309$, respectively).

Methylenetetrahydrofolate reductase genotypes

The proportion of persons in the study with the homozygous normal (CC) genotype for MTHFR was 32% (69 of 215); heterozygous (CT) genotype, 39% (84 of 215); and homozygous mutant (TT) genotype, 29% (62 of 215) (Table 3). The frequencies of the alleles 677C and 677T were 52% (111 of 215) and 48% (104 of 215), respectively. The mean serum tHcy concentration in the adults with the TT genotype was higher (12.30 µmol/L) than in the subjects with non-TT genotypes (CC +CT) (9.89 µmol/L) ($P < 0.001$). Lower serum levels of folate were observed in homozygous mutant adults than in non-TT subjects (23.3 nmol/L vs. 26.5 nmol/L, respectively, $P < 0.001$). A higher proportion of adults with the non-TT genotypes than those with the TT genotype for MTHFR showed tHcy levels < 10 µmol/L (52% and 27%, respectively, $P = 0.002$) (data not shown). Of the 62 TT carriers, 12 of them (19%) presented hyperhomocysteinemia, versus 5 of the 153 non-TT individuals (3%) ($P < 0.001$). No significant differences were found in the mean serum concentrations of folate, vitamin B_{12}, and tHcy between the subjects with CC genotype and those with the CT genotype.

Dietary vitamin intake

In terms of dietary vitamin intake, 77% of the adults reported an adequate intake of folate, and 82% for vitamin B_6. Fewer than 0.5% of the subjects consumed less than one-third of the DRIs for vitamin B_6 and folate. In contrast, 46% of women and 32% of men did not meet two-thirds of the DRI for vitamin B_{12}. In addition, 12% of the adults did not even meet one-third of the DRI for this vitamin.

Geometric means of total homocysteine concentrations

The geometric mean of tHcy concentrations was 27% higher in men (10.43

TABLE 3. Levels of some of the biochemical parameters of a subset (215 persons) of the population in study of determinants of serum homocysteine concentrations, according to their methylenetetrahydrofolate reductase (MTHFR) genotype, Costa Rica, 2000

Indicator	MTHFR genotype					
	Normal (CC) (n = 69)	Heterozygous (CT) (n = 84)	P value[a]	Homozygous (TT) (n = 62)	Non-TT (CC+CT) (n = 153)	P value[a]
Folate (nmol/L) (mean ± SD)[b] (95% CI)[c]	27.2 ± 4.3 (26.3–28.3)	26.0 ± 4.3 (25.2–27.1)	0.107	23.3 ± 4.8 (22.2–24.6)	26.5 ± 4.3 (26.0–27.3)	0.001
Vitamin B_{12} (pmol/L) (mean ± SD) (95% CI)	250 ± 89.0 (229–271)	276 ± 139.0 (246–306)	0.184	254 ±114.0 (227–285)	264 ± 120.0 (245–284)	0.562
Total homocysteine (µmol/L) (mean ± SD) (95% CI)	9.80 ± 2.30 (9.24–10.36)	9.96 ± 2.10 (9.50–10.43)	0.657	12.30 ± 4.80 (11.10–13.50)	9.89 ± 2.20 (9.54–10.25)	0.001

[a] P value according to Mann-Whitney test.
[b] SD = standard deviation.
[c] 95% CI = 95% confidence interval.

µmol/L) than in women (8.21 µmol/L) ($P < 0.001$), and it was 8% higher in the subjects with serum creatinine levels ≥ 88.4 µmol/L ($P = 0.011$) (Table 4). The tHcy concentrations were similar in the two studied age groups (20–29 y and 30–40 y). After multivariate adjustment for age and gender, the geometric mean tHcy concentration was 17% higher in the lowest quartile of serum vitamin B_{12} than in the highest quartile ($P < 0.001$). Similarly, a 16% difference was found between the lowest and the highest quartiles for serum folate ($P < 0.001$). The geometric mean tHcy concentration in coffee drinkers was 5% higher than in nondrinkers (9.64 µmol/L versus 9.14 µmol/L; $P = 0.047$).

No significant differences were found in the tHcy concentrations between the smokers and nonsmokers, and the alcohol drinkers and nondrinkers, or among the different quartiles of dietary vitamin intake (data not shown).

Linear and logistic regression models

According to the linear regression model (Table 5), the main predictors of the serum levels of tHcy were the serum levels of vitamin B_{12} and folate, gender, and the presence of the TT mutation in the MTHFR gene. This model explained 34% of the variance of the tHcy concentrations in the population.

Excluding the nonsignificant variables (age, dietary vitamin intake, coffee drinking, and smoking), a second model (Table 6) established that the tHcy levels were 14.5% higher in men than in women, and 13.6% higher in subjects with the TT genotype than in the non-TT adults. Additionally, this model indicated that a 1% reduction in the serum levels of vitamin B_{12} and folate increased the tHcy levels 20.1% and 35.4%, respectively, keeping the rest of the variables constant.

The inclusion of the natural logarithm of folate, vitamin B_6, and vitamin B_{12} intakes in a third linear regression model (not shown) explained an additional 3% of the variance of tHcy compared to the second model ($R^2 = 0.307$ for the third model vs. $R^2 = 0.282$ for the second model).

TABLE 4. Geometric mean of total serum homocysteine concentration, with 95% confidence interval (95% CI), for suspected and established determinants of total homocysteine concentrations, after adjustment for age and sex, Costa Rica, 2000

Variable	Total homocysteine (µmol/L)		
	Mean	95% CI	P^a
Gender			
Men ($n = 186$)	10.43	10.06 – 10.82	
Women ($n = 213$)	8.21	7.94 – 8.48	0.001
Age (years):			
20 to 29 ($n = 163$)	9.18	8.85 – 9.53	
30 to 40 ($n = 236$)	9.33	9.03 – 9.63	0.490
Vitamin B_{12} (pmol/L):			
< 181 ($n = 95$)	10.17	9.70 – 10.66	
181–240 ($n = 101$)	9.48	9.05 – 9.92	0.029
241–317 ($n = 101$)	8.75	8.36 – 9.16	0.001
≥ 318 ($n = 102$)	8.70	8.31 – 9.11	0.001
Folate (nmol/L):			
< 24.2 ($n = 97$)	10.48	10.00 – 10.97	
24.2–27.0 ($n = 94$)	9.04	8.62 – 9.49	0.001
27.1–29.7 ($n = 105$)	8.58	8.19 – 8.97	0.001
≥ 29.8 ($n = 103$)	9.02	8.61 – 9.45	0.001
Creatinine (µmol/L):			
< 88.4 ($n = 302$)	9.09	8.83 – 9.36	
≥ 88.4 ($n = 97$)	9.82	9.32 – 10.34	0.011
Coffee consumption:			
Nondrinkers ($n = 94$)	9.14	8.89 – 9.41	
Drinkers ($n = 305$)	9.64	9.19 – 10.13	0.047
Cigarette consumption:			
Nonsmokers ($n = 328$)	9.18	8.69 – 9.70	
Smokers ($n = 71$)	9.27	9.01 – 9.54	0.751

[a] Statistical significance according to analysis of variance, comparing with the first, lower, or lowest category or quartile.

The binary logistic regression model (Table 7) established that the risk of having hyperhomocysteinemia was 3.13 times as high in the TT subjects as in the non-TT ones. The risk of having high tHcy decreases 3% for each 10-pmol increase in serum vitamin B_{12}. Serum folate levels and gender were not significant predictors of high tHcy concentrations in the population.

DISCUSSION

The prevalence of hyperhomocysteinemia (that is, >15 µmol/L) in the Costa Rican population studied (6%) is similar to the one estimated for the general population of the United States (5%) (16). In young Asian-Indian residents in the United State

this prevalence is 26% for men and women (17), and the prevalence is 77% for men and women in the country of India (18). There are few studies from around the world that have been done on young healthy adults (20–40 y) and their serum tHcy concentrations, thus making comparisons difficult. Nevertheless, the geometric means of tHcy concentrations for the Costa Ricans in our study were slightly higher than the ones reported in the few works done on subjects less than 40 years old (19, 20).

Contrary to other studies (19, 21), in our study in Costa Rica no relationship was found between age and tHcy concentrations. That was probably because the selected population was relatively young (20 to 40 y). Increases of 1 µmol/L in the tHcy concentration

TABLE 5. Linear regression analysis (Model 1) using the natural logarithm of the serum total homocysteine concentrations as the dependent variable in study of determinants of serum homocysteine concentrations, Costa Rica, 2000[a]

Independent variable	β[b]	P
Gender[c]	0.205	0.002
Age	0.002	0.969
Ln[d] serum vitamin B_{12}	−0.309	0.001
Ln serum folate	−0.267	0.001
Ln intake B_6	0.110	0.144
Ln intake B_{12}	−0.099	0.158
Ln intake folate	0.005	0.928
Coffee drinker[e]	−0.084	0.276
Smoker[f]	0.048	0.441
TT mutation[g] in gene of MTHFR[h]	0.222	0.001

[a] The variables included in this model explained 34% of the variance in the total homocysteine levels in the studied population.
[b] β = slope of the line for the population studied.
[c] For gender, men = 1, women = 0.
[d] Ln = natural logarithm.
[e] Coffee drinker = 1, nondrinker = 0.
[f] Cigarette smoker = 1, nonsmoker = 0.
[g] TT = 1, non-TT = 0.
[h] MTHFR = methylenetetrahydrofolate reductase enzyme.

TABLE 6. Linear regression analysis (Model 2) using the natural logarithm of the serum total homocysteine concentrations as the dependent variable in study of determinants of serum homocysteine concentrations, Costa Rica, 2000[a]

Independent variable	β[b]	P
Ln[c] serum vitamin B_{12}	−0.201	0.001
Ln serum folate	−0.354	0.001
Gender[d]	0.145	0.001
TT mutation[e] in gene of MTHFR[f]	0.136	0.001

[a] The variables included in this model explained 28% of the variance in the total homocysteine levels in the studied population.
[b] β = slope of the line for the population studied.
[c] Ln = natural logarithm of the serum concentrations of vitamins.
[d] Men = 1, women = 0.
[e] TT = 1, non-TT = 0.
[f] MTHFR = methylenetetrahydrofolate reductase enzyme.

TABLE 7. Binary logistic regression model using the serum levels of tHcy < 10 µmol/L or ≥ 10 µmol/L as the dependent variable in study of determinants of serum homocysteine concentrations, Costa Rica, 2000

Independent variable	OR[a]	P
Gender[b]	1.6356	0.1445
Age	0.9926	0.7730
TT mutation[c] in MTHFR[d] gene	3.1287	0.0017
Serum vitamin B_{12} (pmol/L)	0.9971	0.0048
Serum folate (nmol/L)	0.9979	0.9779

[a] OR = odds ratio.
[b] For gender, men = 1, women = 0.
[c] TT = 1, non-TT = 0.
[d] MTHFR = methylenetetrahydrofolate reductase.

have been observed for each decade between 40 and 70 years (22). Furthermore, it has been suggested that high tHcy concentrations in older subjects could be related to an age-associated decrease in the activity of the enzymes involved in homocysteine metabolism or in renal function (23).

The higher serum tHcy concentrations in men than in women that we found in Costa Rica are consistent with the results of other research, suggesting that sex hormones could play an important role in tHcy metabolism (23). High levels of tHcy have been reported in menopausal women who are not undergoing estrogen replacement therapy, while those treated with these hormones have lower serum tHcy concentrations (24). In addition, other research (25) has found an association between creatinine levels and high tHcy concentrations in men, but not in women. This has been explained in terms of body composition, since men have higher muscle mass than women do.

Several lifestyle variables have been associated with hyperhomocysteinemia. In the cohort work with the de-scendants of the Framingham Study (20) and of the Hordaland Study (21), a positive association was observed between smoking and the serum tHcy concentrations. Likewise, several studies have reported a positive association between alcohol consumption and serum tHcy concentrations (2). In our Costa Rica study the serum tHcy concentrations were found to be associated with coffee consumption, in accord with previous reports (20, 21, 26). No significant relationship was observed between tHcy concentrations and other lifestyle variables.

In our study the intake of folate and of vitamin B_6 both showed weak positive correlations ($r < 0.2$) with serum tHcy concentrations; however, the associations disappeared after multivariate adjustment for age and gender. In contrast, Saw et al. (26) and de Bree et al. (2) have reported a strong inverse association between folate ingestion and tHcy concentrations. Although 24-hour recall has demonstrated a good correlation with other dietary assessment methods in nutritional epidemiology (20), in our study it was unable to adequately assess the daily intake of folate, vitamin B_6, and vitamin B_{12}. Nevertheless, the intake data for these three vitamins together explained around 3% of the variance in the serum tHcy levels, according to our third linear regression model.

In both our work in Costa Rica and in other epidemiologic studies (20, 22, 23) an inverse correlation between the serum concentrations of some vitamins (especially folate and B_{12}) and tHcy was found. Data from the Framingham Study (23) indicated that 67% of the subjects with suboptimal levels

of one or more of these vitamins presented high tHcy levels. Folate, in the form of 5-methyltetrahydrofolate, has been considered the most important dietetic determinant of serum Hcy concentrations (2). Multiple intervention and fortification assays (27) have established that folic acid is the most effective vitamin of the B complex for lowering high levels of homocysteine in apparently healthy subjects, using doses as small as 100–400 µg/daily.

A food fortification program was started by the Ministry of Health of Costa Rica in 1997. In 1996, Kim et al. (28) carried out a study in which they found that 98% of the subjects had folate intakes that were less than the recommended 400 ug/day. In women, the mean plasma folate level was 7.9 (± 2.2) nmol/L, and 36% of them had concentrations of less than 6.8 nmol/L, a clear indicator of folate deficiency. In 1997 the Ministry of Health made the enrichment of wheat flour with folic acid obligatory (29). This was extended to corn flour in 1999, milk in 2001, and rice in 2002. Although the purpose of this supplementation was to reduce the number of children born with neural tube defects (anencephaly, spina bifida), this fortification program probably had an important collateral effect on CVD. Since all the adults in our study were characterized by serum fo-late sufficiency, measuring their serum folate levels was not as useful in predicting elevated tHcy concentrations as would be true in countries that do not fortify food with folic acid (21–23).

The presence of the TT mutation in the gene that codes for the MTHFR enzyme had the highest predictive value for high levels of tHcy in our Costa Rica study population. The prevalence of this genotype was higher (29%) than the level reported in various other countries (2.5%–16%) (3, 18, 26, 30). Only Caucasians (36%) and Asians (40%) have higher prevalences of this homozygous mutation than did the persons in our Costa Rica study (31). It's easy to explain the high prevalence of the TT mutation in the MTHFR gene that we found, given that present-day Costa Ricans represent an ancestral gene combination of some 10–15% African, 30% Amerindian, and 50–60% Caucasian origin. The frequency of both alleles (C and T) is practically the same, so the probability that this point mutation in nucleotide 677 is inherited in a homozygous way is high.

Even though this TT mutation is considered a nonmodifiable factor for hyperhomocysteinemia, it's important to diagnose the individuals with the TT genotype in the population in order to identify the people who could most benefit from nutrition interventions.

The vitamin B_{12} serum level in the population in Costa Rica was the second most important predictor of high tHcy levels, so it may be appropriate to consider the supplementation of food with vitamin B_{12}. In randomized intervention trials assessing the effects of cobalamin in lowering serum tHcy concentrations, reductions of around 7% in the levels of this amino acid have been seen (32). Recent research is giving greater importance to cobalamin levels as a determinant of tHcy concentrations. These studies suggest that enhancing vitamin B_{12} status may significantly decrease tHcy levels in people, especially in those individuals with C677T MTHFR polymorphism (33, 34).

Future research should also evaluate the intake and serum levels of other vitamins that are involved in the metabolic cycle of homocysteine, such as B_6 and B_2. Any effort along these lines is justified, given that for each 1-µmol/L decrease in serum tHcy, cardiovascular risk goes down by 10% (2).

REFERENCES

1. Molgaard J, Malinow MR, Lassvik C, Holm AC, Upson B, Olsson AG. Hyperhomocyst(e)inaemia: an independent risk factor for intermittent claudication. J Intern Med. 1992; 231:272–9.

2. de Bree A, Verschuren WM, Blom HJ, Kromhout D. Association between B vitamin intake and plasma homocysteine concentration in the general Dutch population aged 20–65 y. Am J Clin Nutr. 2001;73(6):1027–33.

3. Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, et al. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation. 1996;93:7–9.

4. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Mathews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nature Genetics. 1995;10:111–3.

5. Boushey CJ, Beresford SAAA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA. 1995;274: 1049–57.

6. Costa Rica, Ministerio de Salud, Departamento de Estadística. Mortalidad por enfermedades del aparato circulatorio. San José: Ministerio de Salud; 1996.

7. Campos H, Mata L, Siles X, Vives M, Ordovas JM, Schaefer EJ. Prevalence of cardiovascular risk factors in rural and urban Costa Rica. Circulation. 1992;85:648–58.

8. ESHA Research. The Food Processor for Windows: nutrition and fitness software, version 6.0 [computer program]. Salem: ESHA Research; 1995.

9. Yates AA, Schlicker SA, Suitor CW. Dietary Reference Intakes: the new basis for recommendations for calcium and related nutrients, B vitamins, and choline. J Am Diet Assoc. 1998;98:699–706.

10. National Committee for Clinical Laboratory Standards. Procedures for the collection of diagnostic blood specimens by venipuncture; approved standard. 4th ed. Wayne: NCCLS; 1991.

11. Henry RJ, Cannon DC, Winkelman JW. Clinical chemistry: principles and technics. 2nd ed. New York: Harper & Row Publishers; 1974.

12. Abbott Laboratories, Diagnostics Division. IMx system: homocysteine, vitamin B_{12} and folate. Abbott Park: Abbott Laboratories; 1998.

13. Jacobsen DW. Homocysteine and vitamins in cardiovascular disease. Clin Chem. 1998;44: 8(B);1833–43.

14. Johnson & Johnson. Dry slide technology. Rochester: Johnson & Johnson; 1996.

15. Miller SA, Dykes DD, Polesky HF. A simple salting out for extracting DNA from human

nucleated cells. Nucleic Acids Res. 1988;16:
1215–20.

16. Hankey GJ, Eikelboom JW. Homocysteine
and vascular disease. Lancet. 1999;354:407–13.

17. Carmel R, Mallidi PV, Vinarskiy S, Brar S,
Frouhar S. Hyperhomocysteinemia and cobal-
amin deficiency in young Asian Indians in the
USA. Am J Hematol. 2002;70(2):107–14.

18. Refsum H, Yajnik CS, Gadkari M, Schneede J,
Vollset SE, Orning L, et al. Hyperhomocys-
teinemia and elevated methylmalonic acid in-
dicate a high prevalence of cobalamin defi-
ciency in Asian Indians. Am J Clin Nutr. 2001;
74(2):233–41.

19. Jacques PF, Rosenberg IH, Rogers G, Selhub J,
Bowman BA, Gunter EW, et al. Serum total
homocysteine concentrations in adolescent
and adult Americans: results from the third
National Health and Nutrition Examination
Survey. Am J Clin Nutr. 1999;69:48–9.

20. Jacques PF, Bostom AG, Wilson PW, Rich S,
Rosenberg IH, Selhub J. Determinants of
plasma total homocysteine concentration in
the Framingham Offspring cohort. Am J Clin
Nutr. 2001;73:613–21.

21. Vollset SE, Refsum H, Tverdal A, Nygard O,
Nordrehaug JE, Tell GS, et al. Plasma total ho-
mocysteine and cardiovascular and noncar-
diovascular mortality: the Hordaland Homo-
cysteine Study. Am J Clin Nutr. 2001;74:130–6.

22. Selhub J, Jacques PF, Rosenberg IH, Rogers G,
Bowman BA, Gunter EW, et al. Serum total
homocysteine concentrations in the Third Na-
tional Health and Nutrition Examination Sur-

vey (1991–1994): population reference ranges
and contribution of vitamin status to high
serum concentrations. Ann Intern Med. 1999;
131:331–9.

23. Selhub J, Jacques PF, Wilson PW, Rush P,
Rosenberg IH. Vitamin status and intake as
primary determinants of homocysteinemia
in an elderly population. JAMA. 1993;270:
2693–8.

24. Van der Mooren MJ, Wouters MG, Blom HJ,
Schellekens LA, Eskes TK, Rolland R. Hor-
mone replacement therapy may reduce high
serum homocysteine in postmenopausal
women. Eur J Clin Invest. 1994;24:733–6.

25. Bostom AG, Culleton BF. Hyperhomocys-
teinemia in chronic renal disease. J Am Soc
Nephrol. 1999;10:891–900.

26. Saw SM, Yuan JM, Ong CN, Arakawa K, Lee
HP, Coetzee GA, et al. Genetic, dietary, and
other lifestyle determinants of plasma homo-
cysteine concentrations in middle-aged and
older Chinese men and women in Singapore.
Am J Clin Nutr. 2001;73:232–9.

27. Brouwer IA. Low-dose folic acid supplemen-
tation decreases plasma homocysteine con-
centrations: a randomized trial. Am J Clin
Nutr. 1999;69:99–104.

28. Kim MK, Ordovas JM, Selhub J, Campos H. B
vitamins and plasma homocysteine concen-
trations in an urban and rural area of Costa
Rica. J Am Coll Nutr. 2003;22(3):224–31.

29. Instituto Costarricense de Investigación y En-
señanza en Nutrición y Salud, Centro de Reg-
istro de Enfermedades Congénitas. Prevalen-

cia de enfermedades congénitas por provincia
y cantones: Costa Rica, 1987–2000. Tres Ríos:
INCIENSA; 2002.

30. Schneider JA, Rees DC, Liu YT, Clegg JB.
Worldwide distribution of a common methyl-
enetetrahydrofolate reductase mutation. Am J
Hum Genet. 1998;62:1258–60.

31. Franco RF, Araujo AG, Guerreiro JF, Elion J,
Zago MA. Analysis of the 677 C→T mutation
of the methylenetetrahydrofolate reductase
gene in different ethnic groups. Thromb
Haemost. 1998;79:119–21.

32. Homocysteine Lowering Trialists' Collabora-
tion. Lowering blood homocysteine with folic
acid based supplements: meta-analysis of ran-
domized trials. BMJ. 1998;316:894–8.

33. Bailey LB, Duhaney RL, Maneval DR,
Kauwell GP, Quinlivan EP, Davis SR, et al. Vi-
tamin B_{12} status is inversely associated with
plasma homocysteine in young women with
C677T and/or A1298C methylenetetrahydro-
folate reductase polymorphisms. J Nutr. 2002;
132(7):1872–8.

34. Herrmann W, Obeid R, Schorr H, Zarzour W,
Geisel J. Homocysteine, methylenetetrahy-
drofolate reductase C677T polymorphism and
the B-vitamins: a facet of nature-nurture inter-
play. Clin Chem Lab Med. 2003;41(4):547–53.

RESUMEN

**Factores genéticos
y alimentarios y otros
aspectos del estilo de vida
que determinan las
concentraciones séricas de
homocisteína en adultos
jóvenes en Costa Rica**

Objetivo. La elevación de las concentraciones séricas de homocisteína total (tHci) se
considera un factor que influye de forma independiente en el riesgo de sufrir trastor-
nos cardiovasculares. El objetivo del presente estudio ha sido aportar los primeros
datos que jamás se han reunido acerca de la prevalencia de hiperhomocisteinemia y
sus factores determinantes en una población costarricense.
Métodos. Se llevó a cabo un estudio transversal a fin de determinar las concentra-
ciones séricas de tHci, vitamina B12, folato y creatinina, así como la presencia del ge-
notipo TT respecto de la enzima reductasa del metilenotetrahidrofolato (RMTHF).
También se examinaron la ingestión de vitaminas y otros factores de riesgo relacio-
nados con el estilo de vida. Participaron en el estudio, que se realizó en 2000, 399 adul-
tos costarricenses de 20 a 40 años de edad del valle central del país (donde está San
José, la capital). Se realizaron análisis de la varianza en el caso de las variables conti-
nuas, y se aplicó la prueba de ji al cuadrado en el caso de datos categóricos. Se efec-
tuaron pruebas de correlación de Spearman para determinar la asociación entre dis-
tintas variables. Se llevaron a cabo tres análisis de regresión lineal y un modelo
logístico para datos binarios a fin de determinar qué factores servían para pronosticar
las concentraciones de homocisteína en la población estudiada.
Resultados. La prevalencia general de hiperhomocisteinemia (>15 µmol/L) en la po-
blación fue de 6%; en 31% de la muestra se detectaron concentraciones entre 10 y 15
µmol/L; en 29% se halló el genotipo TT respecto de la enzima RMTHF; 18% tuvieron
deficiencia de vitamina B12 (<165 pmol/L), y nadie tuvo bajas concentraciones séricas
de folato (<7,0 nmol/L). No se hallaron asociaciones significativas entre tHci en el
suero, edad, hábitos tabáquicos, el consumo de alcohol o la ingestión de vitaminas.
Conclusiones. Solamente las concentraciones séricas de vitamina B12 y el genotipo
TT respecto de la enzima RMTHF mostraron tener valor pronóstico con respecto a las
concentraciones séricas de tHci en la población costarricense aquí estudiada.

Palabras clave Enfermedades cardiovasculares, homocisteína, estilo de vida, factores de riesgo,
Costa Rica.