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

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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.

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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 *Hinf* 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

	Folate (nmol/L)		Vitamin B_{12} (pmol/L)		
Total homocysteine	< 7.0 (*n* = 0)	≥ 7.0 (*n* = 399)	< 165 (*n* = 70)	≥ 165 (*n* = 329)	*P* value[a]
< 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

	MTHFR genotype					
Indicator	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.