BIOCHEMISTRY

Total Plasma Homocysteine Concentrations in Puerto Rican Patients With Presumptive Atherosclerotic Coronary Disease

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Background. **In Puerto Rico, it has been established that although coronary heart disease is the leading cause of death, the population has a lower incidence of coronary disease than the continental United States. In addition, the severity of the disease is less aggressive in terms of a lower incidence of ventricular tachycardia and sudden death. A factor in the lower incidence of coronary disease in Puerto Rico could be a lower total plasma homocysteine concentration (tHcys) in our population.**

Methods. **We randomly measured tHcys concentrations in seventy-two Hispanic patients who were hospitalized for coronary angiography at the Cardiovascular Center of Puerto Rico and the Caribbean (UPR Division).**

Results. **The mean tHCys concentration in our patient population is similar than that reported for the Framingham study when adjusted by age (11.2 μmol/L vs. 11.8 μmol/L). In the Puerto Rican population, males had a higher tHcys concentration than females but this difference was not statistically significant (10.9 μmol/L vs. 9.4 μmol/L, p=0.09). In addition, we did not see an increase of tHcys concentrations in diabetic patients when compared with non-diabetics (10.1 μmol/L vs. 10.3 μmol/L, p=0.73). Neither we saw a direct correlation between tHcys concentrations and atherosclerosis as measured by coronary angiography (normal=10.9 μmol/L, mild=8.6 μmol/L, moderate=10.9 μmol/L, severe=10.5 μmol/L; ANOVA=0.29).**

Conclusions. **These results suggest that tHcys concentration is not a good predictor of atherosclerotic coronary disease in our patient population.**

In 1969, McCully reported the case of a child with homocystinuria (inborn error in cobalamin metabolism) that exhibited arterial lesions similar to those seen in older patients with atherosclerotic cardiovascular disease (ACD), with the main difference that the plaques from the child did not contain any lipid deposit (1-3). In addition, another patient with cystathione β-synthase (CBS) deficiency had severe arteriosclerotic lesions, similar to those observed in the child with homocystinuria. Since hyperhomocysteinemia was the only common finding in these two hereditary enzyme disorders, McCully suggested that the arteriosclerotic disease was the result of high total plasma homocysteine concentrations (tHcys).

In recent years, the correlation between tHcys and atherosclerosis has been studied with increased interest in the medical community (4-7). Increased tHcys concentrations have been associated with premature arterial disease (8-11), and a recent study Boushey *et al.* showed through a meta-analysis that elevated tHcys levels is an independent risk factor for ACD not related with hyperlipidemia, hypertension, diabetes, or smoking (12). They also calculated that 10% of the population's risk for ACD appears to be attributed to elevated tHcys concentrations (12). With each 5 μmol/L rise in tHcys levels, the risk for ACD increased by 60% for men and 80% for women, which is similar to a cholesterol increase

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of 20 mg/dL (0.5 mmol/L). In the Hordaland study, they found that elevated tHcys concentrations were associated with major components of cardiovascular risk profile such as male gender, old age, smoking, high blood pressure, elevated cholesterol level, and lack of exercise (13). However, not all studies are consistent with the relation between moderate tHcys concentrations and the relative risk for ACD. A recent analysis of Physician's Health Study data revealed a small relative risk for elevated tHcys concentrations of only 1.3 (not significant), that disappeared after adjustments for other variables were made (14,15). Christen *et al.* (16) recently reviewed all major clinical trials related to tHcys and cardiovascular disease. They concluded that results from prospective studies have shown no predictive ability for tHcys in ACD as done by previous cross-sectional and case-control clinical trials (16). Although much work has been done in the North American and European Caucasian population, there is still the need to determine if tHcys is associated with ACD in the Hispanic population.

Total plasma homocysteine is the sum of protein-bound, free-oxidized (symmetric or mixed disulfides), and reduced species in plasma (17), with a range between 5 to 15 μmol/L in healthy subjects (12). Moderate plasma Hcys values between 15 to 30 μmol/L have been related to low nutritional intake of certain vitamins and genetic defects regarding certain essential enzymes (18). High levels of tHcys are associated with endothelial cell damage and an increase in oxidative tension, leading to smooth muscle cell proliferation (19, 20). tHcys accumulation may be caused by a metabolic block in either the degradation of homocysteine to cystathionine or the remethylation of homocysteine to methionine (Figure 1). The first metabolic block is considered the classic form of hyperhomocysteinemia caused by a deficiency in CBS. This enzyme catalyzes the formation of cystathionine from serine and homocysteine. Vitamin B_6 (pyroxidal-5-phosphate) is essential for the conversion of homocysteine to cystathionine, and it was reported recently that low circulating concentrations of Vitamin B_6 confer an increased risk of atherosclerosis independent of tHcys levels (7). Heterozygotes for CBS deficiency have reduced enzyme activity in cultured fibroblasts and mild hyperhomocysteinemia after methionine loading (4). Two independent studies showed reduced fibroblast CBS activity in the majority of ACD patients with mild hyperhomocysteinemia (4,5). However, these observations were not reproduced by two other groups (21). Furthermore, the calculated number of these heterozygotes in the population was too low to account for the total number of observed hyperhomocysteinemia cases in ACD patients (22).

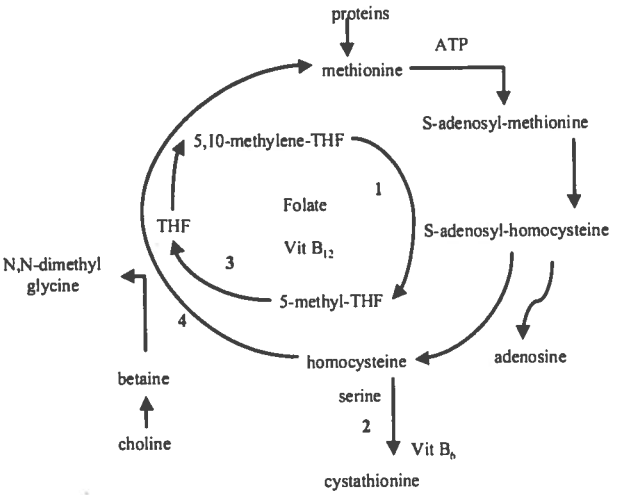

Figure 1. Metabolism of homocysteine. The numbers indicate the most important enzymes involved in the metabolism. 1 = 5,10 methyltetrahydrofolate reductase; 2 = cystathionine β synthase; 3 = methionine synthase; 4 = betaine-homocysteine methyltransferase.

The second metabolic blockage producing hyperhomocysteinemia in ACD patients is in the 5,10-methylenetetrahydrofolate reductase (MTHFR) enzyme, affecting the remethylation process of homocysteine to methionine. Folate and Vitamin B_{12} are essential for this metabolic process, since they couple to donate the methyl group to homocysteine. Low serum folate levels are often associated with high tHcys levels (7, 23, 24) and can increase by the administration of folic acid (25). The reduction of plasma folate levels results from the lack of 5-methylhydrofolate, the product of MTHFR, which is the main form of circulating folate. Thus, it has been recommended the consumption of 400 μg of folic acid/d to decrease ACD risk (26, 27).

In Puerto Rico, it has been established that although coronary heart disease is the leading cause of death, the population has a lower incidence of coronary disease than in the continental United States (28-30). In addition, the severity of the disease is less aggressive in Puerto Rico in terms of a lower incidence of ventricular tachycardia and sudden death (31). Recently, Altieri *et al.* suggested that a possible explanation might be that the Puerto Rican population had a lower LDL/HDL ratio. When compared with the Framingham study, Puerto Ricans had a lower LDL/HDL ratio than in continental United States (2.55 vs. 3.04, respectively $p < 0.001$) (32). Another factor contributing to the lower incidence of coronary disease in Puerto Rico could be a lower tHcys concentration in our population than in the continental United States. In

this study, we report tHcys concentrations from seventy-two patients admitted to the Cardiovascular Center of Puerto Rico and the Caribbean with presumptive coronary heart disease.

Subjects and Methods

Seventy-two individuals were evaluated at Cardiovascular Center of Puerto Rico and the Caribbean (UPR Division) for presumptive coronary heart disease. They were selected from a random sample of patients admitted to the Cardiovascular Center and were divided in four progressive categories: normal, mild, moderate, or severe by two cardiologists after reviewing their coronary angiograms. All patients signed an informed consent form. Information regarding age, gender, smoking habits, diabetes, hypertension, and physical examination was recorded. After an overnight fast, 2 milliliters of blood were obtained and placed immediately on ice. All samples were centrifuged within 2 hours of collection and the plasma was stored at –80°C until tHcys analysis was performed.

Analytical method. Determination of total plasma homocysteine concentration was performed using an HPLC method described previously (33). Briefly, 100 µL of plasma were incubated with dithiothreitol followed by the addition of 1.0 M $HClO_4$ and centrifugation for 2 minutes. The supernatant was neutralized with bicarbonate and Tris HCl (pH 8.0). The sample was then derivatized with 100 mM monobromobimane in the dark at room temperature. The reaction was stopped with $HClO_4$ and the sample was analyzed by HPLC. Hcys levels were determined on a quaternary HPLC system (Hewlett Packard 1050) with a Hypersil C_{18} column and re-injection occurred every 11 minutes. Hcys retention time was 6.5 minutes (Figure 2). The limit of quantitation for plasma total Hcys was 1.3 µmol/L. The intra-day and inter-day coefficients of variation were less than 2% and 10%, respectively.

Statistical analysis. Statistical analysis was performed using Statview® (SAS® Institute Inc., 1998). The Kolmogorov-Smirnov test was used to test for normalcy of distribution. Total plasma Hcys concentrations were normally distributed. Comparisons of tHcys concentrations among study subgroups defined by gender, diabetes, hypertension, smoking habit, and heart condition were performed using two sample t-tests and analysis of variance (ANOVA). Homogeneity of variances among groups was determined using Bartlett's test. Univariate linear regression analysis was performed to study the relation between age and tHcys concentrations. Chi-square was used for comparison of group frequencies, as appropriate. P values less than 0.05 were considered significant.

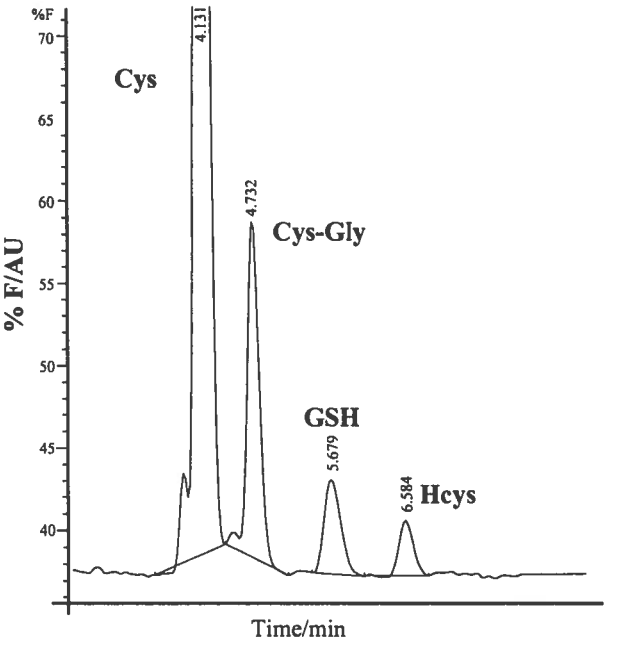

Figure 2. Chromatogram showing the separation of aminothiols for one of the patients. Cys = cysteine; Cys-Gly = cysteine-glycine; GSH = glutathione; Hcys = homocysteine.

Results

Table 1 shows the clinical characteristics of the patient population grouped according the degree of coronary occlusion. The mean age for the group was 61 years with statistical differences between patients with and without coronary artery occlusion (62 y vs. 52 y, p=0.005). The distribution of smokers and hypertensive patients were shifted primary to the patient group with coronary artery occlusion, although this distribution was not statistically

Table 1. Characteristics of the Patient Population

Parameter	Total (n=72)	Normal (n=11)	CHD (n=61)	P value
Age (yrs)	61 ± 11 †	52 ± 10 †	62 ± 11†	0.005 [a]
Smokers	19	3	16	0.72 [b]
Hypertensives	49	6	43	0.72 [b]
Diabetics	35	1	34	0.02 [b]
Coronary angiogram [c]	11,17,13,31	11	17,13,31	

[a] = two sample t-test
[b] = Chi-squared
[c] = Classification was defined by coronary angiography as normal, mild, moderate, severe
† = Mean ± standard deviation

significant. The only distribution that was statistically significant was between diabetic patients, where the majority was allocated in the group with arterial occlusion (34 vs. 1, p=0.02). The distribution of patients according to angiogram results was: no occlusion – 11 patients; mild – 17 patients; moderate – 13 patients; and severe – 31 patients.

Figure 3 shows the distribution of the tHcys concentrations grouped by gender; the mean total concentration for the population was 10.0 ± 3.7 µmol/L. Female patients (9.4 ± 4.1 µmol/L, n=34) had lower tHcys levels than male patients (10.9 ± 3.0 µmol/L, n=38), but this difference was not statistically significant (p=0.09).

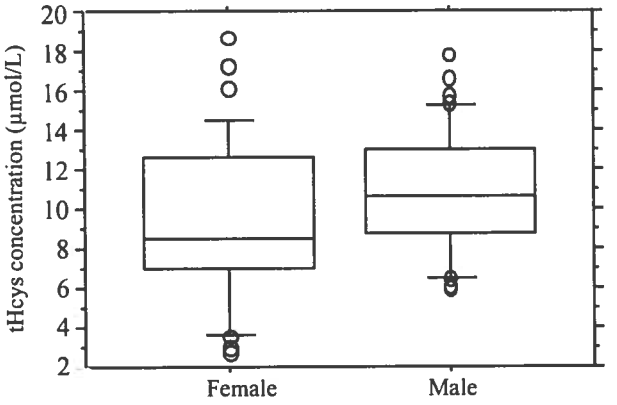

Figure 3. Distribution of total plasma Hcys concentrations in seventy-two Puerto Rican patients with presumptive coronary disease grouped by gender. The tHcys for female patients was 9.4 ± 4.1 µmol/L and 10.9 ± 3.0 µmol/L for male patients. There was no statistical difference between the groups (Student t-test, p=0.09).

Figure 4 shows the distribution of tHycs concentrations in the diabetic patients (n=35) compared with non-diabetic patients (n=37). There were no statistically significant differences in tHcys levels in this population sub-group (10.1 µmol/L vs. 10.3 µmol/L, p=0.73), although most diabetic patients had greater arterial occlusion. We did not observe differences in tHycs concentrations for the other sub-groups: smokers vs. no-smokers (10.5 µmol/L vs. 10.1 µmol/L, p=0.61); or hypertensives vs. normotensives (10.7 µmol/L vs. 9.2 µmol/L, p=0.10).

Figure 5 shows the distribution of tHcys concentrations by status of the artery occlusion. The distribution shows that the patients without occlusion and those with moderate occlusion had the highest tHcys levels (10.9 µmol/L for both groups), followed by the patients with severe occlusion (10.5 µmol/L) and finally patients with mild occlusion (8.6 µmol/L). The ANOVA test did not show a statistical difference in this group of patients.

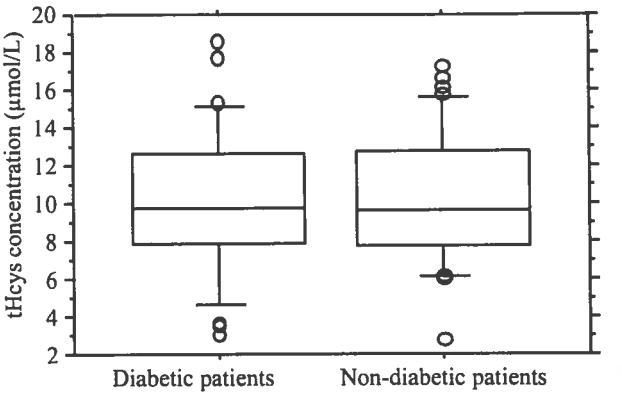

Figure 4. Distribution of total plasma Hcys concentrations in seventy-two Puerto Rican patients with presumptive coronary disease, grouped according to their diabetic condition. The tHcys for diabetic patients was 10.1 ± 3.8 µmol/L and 10.3 ± 3.5 µmol/L for non-diabetics. There was no statistical difference between the groups (Student t-test, p=0.73).

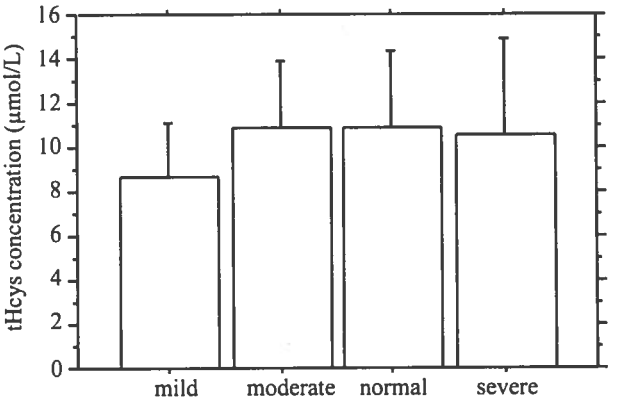

Figure 5. Distribution of total plasma Hcys concentrations in seventy-two Puerto Rican patients with presumptive coronary disease classified according to the degree of artery occlusion. The distributions by groups were as follows: mild = 8.6 ± 2.4 µmol/L; moderate = 10.9 ± 2.9 µmol/L; normal = 10.9 ± 3.3 µmol/L; severe = 10.5 ± 4.3 µmol/L. There was no statistically significant difference between the groups (ANOVA, p=0.29).

Figure 6 shows the regression plot for the relationship between tHcys and age. Although the p value for the regression curve was statistically significant (p=0.03), the regression coefficient (r^2) was only 0.067. Thus, a trend towards an increase in tHcys concentrations with age is present in our population, as observed in other studies.

Discussion

This is the first study evaluating the possible relationship between tHcys concentrations and the incidence of coronary heart disease in the Puerto Rican population.

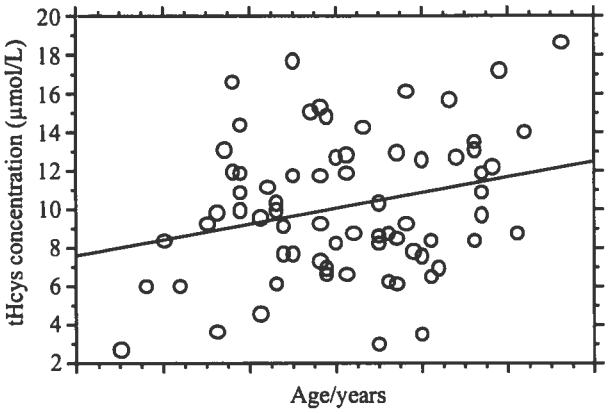

Figure 6. Distribution of total plasma Hcys concentrations vs. age. The equation describing the complete range is y = 0.082 x + 5.142 with a regression coefficient (r^2) of 0.067 (p=0.03)

When compared to other populations from around the world, we did not see major differences in tHcys concentrations. Our mathematical mean was similar to that reported in the Norwegian population (10.0 µmol/L vs. 11.0 µmol/L, respectively) (13). In addition, our group had no different tHcys concentrations to the values reported by the Framingham study when adjusted by age (11.2 µmol/L vs. 11.8 µmol/L, respectively). Moreover, our results compared favorably with the German population, since we did not observe substantial differences among these two groups (10.0 µmol/L vs. 9.2 µmol/L, respectively) (34). Similarities also apply when tHcys concentrations are compared by gender in the aforementioned studies. The results presented by our group are similar to those reported in the United States children, where the Hispanic population had tHcys concentrations equivalent to that of the Caucasian population (35). Thus, the differences observed in the lower incidence of ACD in the Puerto Rican population when compared to the United States should be attributed to factors other than moderate or elevated tHcys concentration.

In the diabetic population, we did not find differences in tHcys concentration when compared to non-diabetic patients. Thus, tHcys does not play a significant role in the genesis of ACD in this patient population. Preliminary results from our group suggest that the plasma glutathione/homocysteine ratio might be an important surrogate marker to describe ACD in the diabetic population rather than tHcys concentrations (data not shown).

We obtained unexpected results when the participants were grouped according to the degree of artery occlusion. We anticipated to find the highest tHcys concentrations in those patients with moderate or severe arteriosclerotic disease. However, tHcys concentrations were highest in patients with no occlusion and moderate occlusion, whereas the patients with mild occlusion had the lowest tHcys level. This result was even more surprising, since the patients with no occlusion were much younger than the other patient sub-groups. It has been shown by this and other studies that tHcys concentrations increase with age. We conclude that according to our results, the tHcys concentration is not a reliable predictor of ACD in our patient population.

Resumen

En Puerto Rico, se ha comprobado que a pesar de que la enfermedad coronaria es la principal causa de muerte, la población tiene una incidencia menor de estas enfermedades que en los EEUU y posee una incidencia menor de taquicardia ventricular y muerte súbita. Se postuló que el factor que puede contribuir a una menor incidencia de enfermedades coronarias en Puerto Rico sea que las concentraciones totales de homocisteina plasmática (tHcys) en nuestra población sean menores que en la población de los EEUU. En este estudio se midió la concentración de tHcys en setenta y dos pacientes hispanos hospitalizados para angiografía coronaria en el Centro Cardiovascular de Puerto Rico y el Caribe. La concentración promedio de tHcys en estos pacientes resultó similar a la reportada por el estudio de Framingham cuando es ajustada por edad (11.2 µmol/L vs 11.8 µmol/L). En la población puertorriqueña, los varones tenían una concentración mayor de tHcys que las mujeres (10.9 µmol/L vs 9.4 µmol/L, p = 0.09), aunque esta diferencia no fue estadísticamente significativa. Además, no observamos un aumento en la concentración de tHcys en pacientes diabéticos cuando se comparan con los pacientes no diabéticos (10.1 µmol/L vs 10.3 µmol/L, p = 0.73). Tampoco observamos una correlación directa entre la concentración de tHcys y otras condiciones cardiacas encontradas por angiografía coronaria (normal = 10.9 µmol/L, leve = 8.6 µmol/L, moderado = 10.9 µmol/L, severo = 10.5 µmol/L; ANOVA = 0.29). Estos resultados sugieren que la concentración de tHcys no es un buen indicador para la enfermedad arterioesclerótica coronaria en nuestra población de pacientes cardiacos.

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