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PREVALENCE OF FOLATE DEFICIENCY AND HYPERHOMOCYSTEINEMIA IN A DEVELOPING COUNTRY: RESULTS FROM A LARGE POPULATION STUDY IN VENEZUELA

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ABSTRACT. To evaluate the prevalence of hyperhomocysteinemia in apparently healthy individuals and its relationship to the classical cardiovascular risk factors and nutritional and genetic determinants in a developing country a randomized cross-sectional study was made in the Venezuelan population. Apparently healthy subjects (N = 3400; 9 - 60 yr) recruited, on a voluntary basis, from urban and rural areas in 9 states of the country which gather more than 60% of the total population. A Clinical and anthropometric evaluation, fasting plasma glucose, creatinine, blood lipids, fibrinogen, C-reactive protein, plasma total homocysteine (tHcy), folic acid and vitamin B12 were done in all subjects. The C677 →T polymorphism of MTHFR was evaluated in a random sub-sample of the mixed-blood population (N = 535) and in a sample of Venezuelan blacks (N = 115). Final analysis was done on 3062 subjects (1608 females, 1454 males). An important deficiency in plasma folate was found (~ 50% of recommended value), in 86.0% of the population. Mean value for tHcy stays below 10.5 µmol/L for both genders in all age ranges studied. Hyperhomocysteinemia (> 12 µm/L) is found in 12% of females and 26% of males. MTHFR polymorphism reflects the spanish genic penetration. There is no association between hyperhomocysteinemia and the polymorphism. Hyperhomocysteinemia in our population is mainly related to marked deficiency in folic acid. The relatively lower mean values of tHcy, compared to developed countries, seem related to a general nutritional deficit. **Key words**: Homocysteine, Folic acid, Hyperhomocysteinemia, MTHFR polymorphism, Methylenetetrahydrofolic acid Reductase, developing country.

PREVALENCIA DE DEFICIENCIA DE FOLATO E HIPERHOMOCISTEINEMIA EN UN PAÍS EN DESARROLLO: RESULTADOS DE UN ESTUDIO POBLACIONAL REALIZADO EN VENEZUELA.

RESUMEN. La prevalencia de hiperhomocisteinemia y su relación con determinantes genéticos, nutricionales y factores clásicos de riesgo cardiovascular, fue evaluada a través de un estudio epidemiológico, al azar, de tipo corte transversal, en una muestra razonablemente representativa de la población en Venezuela para el período del estudio. Se evaluaron sujetos aparentemente sanos (n = 3400; de 9 a 60 años de edad), provenientes de áreas rurales y urbanas de 9 estados del país. A todos los sujetos se les realizó una evaluación clínica y antropométrica y se les determinó glucosa sanguínea en ayunas, perfil lipídico, creatinina, fibrinógeno, proteína C reactiva, homocisteína total plasmática (tHcy), ácido fólico, vitamina B12. El polimorfismo MTHFR C677 T fue evaluado en una muestra al azar total de 650 sujetos (Mestizo = 535; Negros = 115). La muestra total analizada fue de 3062 sujetos (Femeninos = 1608; Masculinos = 1454). Se encontró una importante deficiencia de ácido fólico (86% de la población tiene valores cercanos al 50% del valor de folato recomendado). EL valor promedio de tHcy fue menor de 10,5 µM para ambos sexos en todos los grupos etarios evaluados. La prevalencia de hiperhomocisteinemia (>12 µM) fue de 12% en mujeres y 26% en hombres. El polimorfismo MTHFR C677 T, refleja la penetración génica española. La prevalencia de hiperhomocisteinemia en la población venezolana está principalmente relacionada con la deficiencia de ácido fólico. El menor

INTRODUCTION

During the last decade, interest in homocyst(e)ine and its potential role as an independent risk factor for cardiovascular disease resulted in numerous publications reporting results from *in vitro* as well as *in vivo* and epidemiological studies that in general, support the independent relationship of mild hyperhomocysteinemia and cardiovascular disease [7,13]. However, relevant epidemiological studies have been made in developed countries[8,9,15, 27,34,38,39] while population data from developing countries are, to our knowledge, almost inexistent. Determinants of plasma total homocysteine (tHcys) are numerous [35], however, for presumed healthy individuals, the most relevant are age, gender and nutritional status. Data from seven large population studies[35], suggest that the upper reference values for fasting tHcys in developed countries, in children < 15 years is 8 µmol/L (folate supplemented) and 10 µmol/L (no supplemented), while in adults 15 - 65 years is 12 µmol/L (folate supplemented) and 15 µmol/L (no supplemented). As stated above, no such information is available for developing countries. The present paper reports results from a large epidemiological survey that evaluated plasma tHcy, folate and vitamin B_{12} in a sample of the general population in Venezuela.

SUBJECTS AND METHODS

Study sample:

Apparently healthy subjects (9 to 60 years) were recruited between September 2000 and June 2004 (cross-sectional study), on a voluntary basis, with the help of the recognized community organizations. Community leaders were given oral and written information on the objectives of the study and were asked to pass the information to their respective communities. Subjects were recruited from at least one city and one rural town in the following states: Aragua, Miranda, Lara, Falcón, Carabobo, Anzoátegui, Sucre, Yaracuy and the Metropolitan District which include

the capital city, Caracas. These states together gather more than 60 % of the total country population. Although, due to ethical considerations, the protocol design was done on a voluntary basis, care was taken in order to keep the appropriate proportion corresponding to the total population in each geographical area studied. All the subjects that voluntarily accepted to participate in the study received written information detailing the objectives and the sampling protocol as well as a separate form of Valid Informed Consent. A special form of the later was prepared for children and adolescents younger than 18 years of age. In this form, besides their legal representative consent, the subject consent was also required. Exclusion criteria: a) established athero-thrombotic disease, b) Diabetes, c) hypertension, d) infectious or inflammatory diseases and, e) pregnancy. A total of 3400 subjects were recruited, 338 were excluded from the final analysis due to a) incompleteness of data or b) the exclusion criteria. From the 3062 subjects that comply, 1608 (52.5 %) were feminine and 1454 (47.5 %) masculine. The study protocol, as well as the Valid Informed Consent forms were evaluated and approved by the Bioethics Institutional Committee.

Blood samples were obtained from the antecubital vein, under aseptic conditions, by well trained personal. Sample (10 mL) was divided in: a) 6.0 mL for plasma preparation (EDTA as anticoagulant) and b) 4 mL for serum preparation (no anticoagulant). Samples for plasma were kept at 4 °C for not more than 30 minutes and were centrifuged at 4 °C at 2000 xg for 30 minutes (Sorvall RC3). Samples for serum were incubated at 37 °C for 20 minutes and serum obtained after centrifugation at 2000 xg for 30 minutes. The collected plasma, serum and buffy coat from the anticoagulated blood were immediately placed on dry ice for transport to the laboratory. All samples were kept at -80 °C until processed. Subjects were asked to fast overnight and sampling was done between 6 AM and 8:30 AM, depending on the number of subjects.

Each subject was given a physical examination comprising blood pressure, weight, height, hip and waist circumference and a clinical history which included pathological antecedents (personal and familial), medication, vitamin supplements, tobacco use and physical activity.

Biochemical parameters

Fasting plasma glucose, creatinine, total cholesterol, VLDL-cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, fibrinogen, C-reactive protein, plasma total homocysteine (tHcy), plasma folic acid and vitamin B12. The C677→T polymorphism of the Methylen-tetrahydrofolate Reductase (MTHFR) was evaluated in a random sub-sample (N = 535).

MATERIALS AND METHODS

Plasma total homocysteine was measured by using high performance liquid chromatography with fluorescence detection [18]. Plasma lipoproteins were measured as described by Camejo et al [6]; Lipoprotein cholesterol was measured as described by Bowman et al [4]. Plasma triglycerides concentration was measured as described by Biggs et al [3]. Fibrinogen was measured by the clot-weight method [25], using EDTA as anticoagulant [20]. Plasma C-Reactive Protein was measured as described by Rifai et al [35]. The MTHFR 677C→T mutation was detected by polymerase chain reaction using the primers described by Frosst et al [17]. Plasma Folate and vitamin B12 concentrations were simultaneously measured using the Quantaphase® II folate/B12 radioassay (Bio-Rad, Diagnostic group, CA, USA). Plasma glucose and creatinine concentrations were measured using commercial kits (Chemroy, Biochemical Trade Inc; San Antonio, TX, USA and Laboratorios Biogamma CA, Venezuela, respectively).

Statistical analysis

For analysis, the population studied was stratified by gender and age. The age ranges were: 9 - 14, 15 - 19, 20 - 29, 30 - 44, and 45 - 60 years, respectively. Because most biological parameters have a non-Gaussian distribution, we routinely used the Manly's exponential transform[31] followed by the hyperbolic sine transform [23] on the original data. Results are presented as the mean and 95% confidence interval (CI95), in the original units. Estimation of fractiles and their confidence intervals was done by the parametric method as suggested by the International Federation of Clinical Chemistry [26]. Systat v.10 (SPSS, Inc) was used for chi-square test (x^2) to compare proportions and t-test to compare means.

RESULTS

Table I shows the general characteristics (anthropometric and biochemical) of the study sample (N = 3062, F = 1608 and M = 1454), stratified by gender an age. Body Mass Index (BMI) values in both genders are above the recommended values in the age ranges of 30 - 60 yr. In both cases, an age related tendency to overweight and obesity is observed. In these age ranges, the prevalence of overweight was 36% in females and 43% in males while obesity is 27% in females and 22% in males. In the population sample studied (all subjects), the prevalence of obesity (BMI >30) was 25%. The other parameter that also shows an age related increase, in both sexes, is the plasma concentration of C - reactive protein. In this case, it can be observed that even in the 20 - 29 yr. range, women show concentrations above 2 mg/L, while in men this is only observed in the age ranges above 30 yr. Highest proportion of smokers is seen in women above 30 yr. and in men above 20 yr., respectively. All other parameters fall within the normal range for apparently healthy people. Fibrinogen shows the characteristic gender and age dependence of its plasma concentration.

Table II shows the values (mean and 95% confidence interval) for plasma concentrations of homocysteine, folic acid and vitamin B12 for the population studied, stratified by gender and age-range. The most striking observation, shown in Table II is the consistently low values for the concentration of folic acid, with a mean value for all age-ranges and gender around 7 nmol/L (~3 ng/mL).

It can be seen that homocysteine concentration is higher in males than females, at all age-ranges and shows

TABLE I. Anthropometric and biochemical characteristics of the population studied, stratified by age and gender.

Gender	FEMALES					MALES				
Age range (yr)	**9 - 14**	**15 - 19**	**20 - 29**	**30 - 44**	**45 - 60**	**9 - 14**	**15 - 19**	**20 - 29**	**30 - 44**	**45 - 60**
BMI	**19.5*** 19.1 to19.9	**21.8** 21.4 to 22.3	**23.6** 23.0 to 24.1	**25.8** 25.4 to 26.3	**27.3** 26.8 to 27.7	**18.9** 18.5 to 19.3	**21.5** 21.2 to 21.9	**23.5** 23.2 to 23.9	**26.5** 26.0 to 27.0	**26.5** 25.9 to 27.1
W/H ratio	**0.78** 0.75 to 0.84	**0.79** 0.77 to 0.80	**0.79** 0.78 to 0.80	**0.82** 0.80 to 0.83	**0.84** 0.83 to 0.85	**0.83** 0.82 to 0.84	**0.84** 0.83 to 0.85	**0.86** 0.85 to 0.87	**0.89** 0.88 to 0.90	**0.91** 0.90 to 0.92
Fasting Plasma Glucose (mg/dL)	**74.5** 73.3 to 75.8	**74.3** 73.2 to 75.5	**74.6** 73.3 to 76.0	**76.9** 75.5 to 78.2	**80.6** 78.9 to 82.4	**77.5** 76.2 to 79.0	**77.6** 76.2 to 79.0	**77.0** 75.8 to 78.2	**82.7** 80.8 to 84.7	**83.8** 81.3 to 86.5
Creatinine (mg/dL)	**0.76** 0.72 to 0.81	**0.82** 0.79 to 0.85	**0.86** 0.84 to 0.88	**0.86** 0.83 to 0.89	**0.89** 0.86 to 0.92	**0.76** 0.74 to 0.79	**0.87** 0.79 to 0.94	**0.92** 0.89 to 0.95	**0.97** 0.93 to 1.0	**1.0** 0.95 to 1.04
Cholesterol Total (mg/dL)	**161.1** 157.7to 164.5	**162.5** 158.4 to 166.7	**168.9** 165.2 to 172.7	**183.0** 179.6 to 186.4	**207.2** 203.2 to 211.4	**149.8** 146.6 to 153.1	**148.0** 144.6 to 151.7	**161.5** 158.1 to 165.1	**190.5** 186.0 to 195.1	**198.1** 192.7 to 203.6
VLDL-Chol (mg/dL)	**26.1** 25.7 to 26.5	**24.7** 24.1 to 25.3	**25.9** 25.9 to 26.3	**27.1** 26.7 to 27.5	**28.7** 28.2 to 29.2	**25.0** 24.5 to 25.4	**24.6** 24.1 to 25.1	**26.0** 25.5 to 26.5	**29.1** 28.4 to 29.9	**29.1** 28.4 to 29.9
LDL-Chol (mg/dL)	**90.4** 88.0 to 92.8	**87.5** 84.4 to 90.5	**94.0** 91.2 to 97.0	**106.0** 103.3 to 108.6	**125.2** 121.8 to 128.6	**82.2** 79.9 to 84.6	**78.8** 76.2 to 81.6	**88.5** 85.5 to 91.7	**110.7** 107.2 to 114.3	**119.7** 115.0 to 124.4
HDL-Chol (mg/dL)	**43.7** 42.8 to 44.6	**47.8** 47.4 to 48.2	**47.5** 45.3 to 49.7	**48.6** 47.8 to 49.5	**50.4** 49.4 to 51.4	**42.3** 41.3 to 43.3	**44.7** 43.6 to 45.7	**45.7** 44.8 to 46.6	**46.7** 45.7 to 47.6	**45.6** 44.6 to 46.8
Triglycerides (mg/dL)	**81.5** 77.4 to 85.7	**73.45** 69.0 to 78.2	**84.3** 80.0 to 88.8	**95.6** 91.3 to 100.1	**120.7** 115.1 to 126.5	**72.2** 68.8 to 75.8	**66.5** 62.8 to 70.6	**81.0** 77.2 to 84.9	**130.2** 122.2 to 138.9	**135.4** 124.9 to 146.5
Fibrinogen (g/L)	**2.83** 2.75 to 2.92	**2.92** 2.80 to 3.04	**2.94** 2.83 to 3.04	**2.90** 2.82 to 2.98	**3.17** 3.06 to 3.27	**2.81** 2.72 to 2.91	**2.46** 2.38 to 2.56	**2.48** 2.40 to 2.55	**2.67** 2.58 to 2.77	**2.80** 2.70 to 2.92
C Reactive Protein (mg/L)	**1.64** 1.39 to 1.94	**1.05** 0.91 to 1.23	**2.5** 2.1 to 3.0	**2.5** 2.2 to 2.9	**3.1** 2.7 to 3.6	**1.3** 1.12 to 1.52	**1.35** 1.14 to 1.61	**1.70** 1.49 to 1.96	**2.52** 2.15 to 2.96	**2.27** 1.85 to 2.82
TAS (mm Hg)	**98.5** 97.1 to 99.9	**106.7** 104.9 to 108.1	**108.6** 107.2 to 110.2	**115.5** 114.1 to 117.8	**123.6** 121.6 to 125.6	**100.0** 98.2 to 101.0	**111.0** 109.6 to 112.4	**116.6** 115.4 to 117.7	**120.0** 118.6 to 121.5	**124.1** 121.9 to 126.3
TAD (mm Hg)	**63.2** 62. to 64.3	**69.9** 68.6 to 71.2	**71.4** 70.3 to 72.5	**74.9** 73.8 to 76.0	**79.3** 78.2 to 80.5	**62.3** 61.3 to 63.2	**68.2** 67.1 to 69.4	**73.1** 72.1 to 74.1	**77.9** 76.8 to 78.9	**80.5** 79.0 to 82.
Smokers (%)	2.4	2.5	7.5	19.7	18.6	1.6	22	28.3	26.5	22.9
N	286	199	279	446	398	319	259	378	306	192

*Mean, CI 95%

TABLE II. Plasmatic Homocysteine, Folic acid and Vitamin B12 of the population studied, stratified by age and gender.

Gender	FEMALES					MALES				
Age range (yr)	**9 - 14**	**15 - 19**	**20 - 29**	**30 - 44**	**45 - 60**	**9 - 14**	**15 - 19**	**20 - 29**	**30 - 44**	**45 - 60**
tHcy (µmol/L)	**6.92*** 6.69 to 7.15	**7.23** 6.87 to 7.60	**7.82** 7.51 to 8.14	**8.04** 7.79 to 8.30	**8.72** 8.43 to 9.04	**7.14** 6.90 to 7.39	**9.05** 8.62 to 9.52	**9.70** 9.33 to 10.09	**10.21** 9.82 to 10.63	**9.89** 9.43 to 10.38
Folic Acid (nmol/L)	**7.43** 6.93 to 7.95	**6.67** 6.24 to 7.16	**6.27** 5.86 to 6.73	**6.15** 5.83 to 6.49	**6.88** 6.51 to 7.28	**7.23** 6.87 to 7.61	**6.43** 6.08 to 6.82	**5.89** 5.61 to 6.18	**5.61** 5.27 to 6.0	**5.87** 5.41 to 6.4
Vit. B12 (pmol/L)	**305.13** 283.75 to 328.99	**304.09** 282.10 to 328.86	**275.63** 257.10 to 296.28	**305.4** 285.6 to 327.3	**327.3** 305.7 to 351.3	**308.46** 289.35 to 329.45	**319.79** 297.44 to 344.87	**283.61** 267.33 to 301.45	**286.6** 266.12 to 309.42	**309.75** 280.14 to 344.3
N	286	199	279	446	398	319	259	378	306	192

*Mean, CI 95%

the characteristic increase with age in both genders. It is worth noting that the highest homocysteine value is seen in the masculine age range 30 - 44 yr., and corresponds also to the lowest folic acid concentration. The mean value for vitamin B12 for both sexes and all age ranges are well within the recommended values (>180 pmol/L). In a relatively small sub-sample (N = 300), the mean value for vitamin B6 was above 70 nmol/L (data not shown).

There is an inverse relationship of homocysteine concentration with both, plasmatic folic acid and vitamin B12. Linear regression of the log transformed data for tHcy vs folic acid and vitamin B12 yields a negative slope (-0.13 ± 0.01 and -0.07 ± 0.01 for folic acid and vitamin B12, respectively; $p < 0.0001$ for both). This inverse association was also found at all age's ranges and in both genders (data not shown). The prevalence of folic acid deficiency in the population studied, defined as the percentage of subjects with plasmatic folic acid values below the FAO/WHO recommendation (12 nmol/L) [11,24], amounts to 86.0%. Although noticeable, the inverse association of tHcy concentration with the concentration of vitamin B12 seems less important than that with folate.

Table III shows the mean (CI95%) and the 95th percentile (CI90%) for homocysteine from the relatively small subpopulation that comply with the recommended values for folic acid and vitamin B12 (vitamin replete) (14.0%). 34.3% of this subpopulation reported regular ingestion of polyvitamin pills, while in the subgroup that do not comply, only 8% reported regular ingestion of a vitamin supplement. Due to the small number of subjects, the sample was only stratified by sex, and the values were age-adjusted for each gender category. In both genders, there is a statistically significant difference between means and between the values corresponding to the 95th percentile ($p < 0.001$). As expected, lower values for both parameters are found in the "vitamin replete" group.

The sex-specific 95th percentile values from the "vitamin replete" group were used to estimate the prevalence of hyperhomocysteinemia at each age-range, for the population studied (Table IV). This table also shows, for the sake of comparison, the prevalence of high homocysteine concentration based on the commonly used 12 µmol/L cutoff [22]. As can be seen, the prevalence of hyperhomocysteinemia in males almost duplicate that in females, independently of the cutoff used. The prevalence also increases with age. While in women, only in the age-range of 45-60 yr the prevalence of hyperhomocysteinemia attains > 20%, in males, values above 27% are found already in the 15-20 yr range and attain >30% above 20 yr.

Table V shows the distribution of the genotype frequencies for the MTHFR C677→T polymorphism. Mutation was evaluated in a random sample from the total population (N = 535). For comparison, the frequency of polymorphism was also evaluated in 115 subjects of African origin, whose ancestors established in Venezuela at the beginning of the XVII century. As shown in the table, the combined prevalence of CT+TT in the mixed-blood (mestiza) sample (N = 535) amounts to 38.7 %, corresponding to 27.8 % CT and 10.9 % TT. In contrast, in the sample from subjects of African origin (N = 115) only the CT polymorphism was found, with a prevalence of 12.2 % ($p < 0.001$, x^2, ± = 0.05). However, the prevalence of hyperhomocysteinemia is very similar in all groups ($p = > 0.4$, x^2, ± = 0.05). Level of plasma folic acid and vitamin B12 were similar in both sub-groups (not shown).

Table III. Total plasma homocysteine (µM) concentration for subjects that comply with WHO recommended values for plasma folic acid (> 12 nmol/L),compared to non compliant subjects (< 12 nmol/L).

Non Compliant	N	Mean (CI95)	95th Percentile (CI90)
Female	1375	**8.52** (8.36 to 8.69)	**13.07** (13.05 to 13.08)
Male	1258	**8.70** (8.52 to 8.88)	**13.96** (13.94 to 13.97)
Compliant			
Female	233	**7.52** (7.20 to 7.86)	**11.59** (11.53 to 11.66)
Male	196	**7.33** (7.01 to 7.67)	**11.21** (11.15 to 11.28)
P*		< 0.001	< 0.001

Table shows the mean (CI95), and the 95th percentil (CI90), stratified by gender. * Non Compliant vs Compliant, unpaired t-Test, α=0.05

Table IV. Prevalence of high plasma homocysteine concentration in the population studied, stratified by age-range and gender.

	Females			Males		
Age range	**n**	**A %**	**B %**	**n**	**A %**	**B %**
9 to 14	286	4.55	3.5	319	8.15	7.21
15 to 19	199	9.05	7.04	259	27.03	22.78
20 to 29	279	12.90	11.83	378	31.75	27.51
30 to 44	446	11.44	8.97	306	35.29	31.05
45 to 60	398	20.10	18.34	192	34.38	30.21
total	1608	12.31	10.57	1454	26.82	23.31

Column A = Prevalence estimated from the 95th percentile of the «vitamin replete» group (11.59 µmol/L and 11.48 µmol/L for females and males, respectively).Column B = Prevalence estimated from the 12 µmol/L international cutoff.

Table V. Prevalence of the Methylentetrahydrofolate Reductase (MTHFR) C677→T polymorphism in the population studied.

Population	**N**	**CC (%)**	**CT (%)**	**TT (%)**
Mixed (mestiza)	535	61.2 (11.2**)	27.8* (7.4**)	10.9 (6.7**)
African Origin	115	87.8 (7.9**)	12.2* (7.1**)	0.0

* $p < 0.001$; ** $p > 0.4$; x^2 (α=0.05)
In parenthesis the prevalence of homocysteine concentration above 12 µmol/L, in each category.

DISCUSSION

The present study describes the results of a large population evaluation of the concentrations of plasma homocysteine, folic acid and vitamin B12 in a developing country, in apparently healthy subjects. These biochemical parameters are good markers of the general nutritional condition of the population and, in the case of homocysteine is considered one of the modifiable cardiovascular risk factors, amenable of correction via simple and economical public health policies. The study sample can be considered representative of healthy adolescents and adults in Venezuela. It should be noted that we did not use ethnical stratification because our population is a mixed blood population (mestiza), with the following mixture percentages: Spanish, 0.5 - 0.7; African, 0.08 - 02; Amerindian, 0.2 - 0.3, based on the distribution of

RH and ABO groups [36]. As can be seen in Table I, most biochemical parameters lie within normal ranges, although values for C-reactive protein in the age ranges above 20 yr in women and 30 yr in men are at the critical level which is considered of significance for cardiovascular risk [12,44]. Similar trend was observed in the Dallas Heart Study, where women had higher CRP levels than males[29]. The high proportion of obesity observed (25% for the entire population evaluated) is probably related to the carbohydrate-rich diet prevailing in our population[30]. Similar results have been recently reported from a large population study in the state of Zulia, in the northwest part of the country [12].

It is somehow surprising that the mean values for the plasma concentration of homocysteine in the population under study, in the >18 yr age-range is 8.9 µmol/L, well below what one would expect from the mean values for plasma folic acid concentration of 6.17 nmol/L, which is about 50 % of the minimum recommended concentration (12 nmol/L)[11] for 86.9 % of the population evaluated. Such high prevalence of folic acid deficiency has also been found in a small sample of a Bari indians community living in the western border of Venezuela [10]. The apparent discrepancy is further supported by the strong inverse relationship between homocysteine and folic acid similar to what has been described by others [39,41]. Also, in a survey conducted in The Netherlands on 101 apparently healthy subjects (e>18 yr), the mean tHcys value found was 9.6 µmol/L with a mean folic acid value of 12 nmoles/L [13]. This apparent discrepancy is probably related to the general nutritional deficit that has been found in our population, which traduces in a low intake of animal proteins and in which, the major components in the diet are based on carbohydrates [30]. The values found for both genders in the age range 9 - 19 years (Table 2) are slightly higher than those reported for the same age range in Poland [21] and in Mexican Americans living in the USA [27]. The small proportion of subjects (14.0 %) that comply with the recommended values for plasmatic folic acid also show lower values for homocysteine, and much lower 95th percentile values. The 95th percentile values of 11.59 µmol/L and 11.48 µmol/L for females and males, respectively, can not be taken strictly as "reference values" due to the small sample number; however, they do point towards the upper limit of desirable homocysteine values for this population, under the current socio-economic status. Even with the limitation mentioned, these values are very close to those found by Selhub et al [38] in the "vitamin replete" group in the 20 - 39 years of age. Based on these "tentative referential values", the estimated prevalence of hyperhomocysteinemia in the population studied is about 12 % in females and 27 % in males. When stratified by age, the prevalence attains >30% in males older than 20 yr, with a peak in the 30-44 yr range. These values are well above those reported in the USA before the mandatory fortification of cereals with folic acid [38] and those recently reported for a population sample from 11 European countries [8]. In this report [8], however, values for plasmatic folic acid or vitamine B12 are not given. If we use the 15 µmol/L cutoff suggested [8], the prevalence of elevated homocysteine in our 20 - 60 years population range falls to 8.3 %, which is still higher than that reported for the European population (< 5.9%). Different nutritional habits among those populations may be responsible for this behavior.

Besides the strong negative correlation already mentioned, of tHcys with folate and vitamin B12, a weak positive correlation was found with BMI (Pearson r = 0.062, $p < 0.001$, ± = 0.05) and WH ratio (Pearson r = 0.093, $p < 0.001$, ± = 0.05), after adjusting for age.

The prevalence of the MTHFR C677→T and TT polymorphisms in the population, as shown in Table 5, is not very different to reports by other investigators [5,16] and, in our case, reflects the genic distribution based on ABO and Rh groups [36]. Also, the 12.2 % prevalence of CT in the black Venezuelan population is comparable to that found in Brazil for African blacks, but lower than that found for Brazilian blacks[2].

The results in Table V also suggest that the major determinant of homocysteine concentration in the population studied is folic acid. A higher frecuency for the 677C'!T and TT polymorphisms in Indian populations living in the northwestern part of Venezuela (Wayuu and Yupkas etnias) have been reported by Vizcaino et al [42,43]. It is interesting the apparent association of the mutations with the homocysteine values found in these studies, as well as the higher values for seric folate which attains a mean value well above 12 nM, in contrast with the low values reported in the present study and in a recent large study (N = >5000) on children, adolescents and pregnant women in Venezuela [19]. Moreover, results from another large study in which homocysteine and folic acid levels were evaluated in 1909 subjects in Maracaibo [1] also show mean values around 8 nM, well below the recommended OMS value. As discussed above, such high values were found by us in only 14% of the population studied.

The results described represent, to our knowledge, the largest population study on the concentration of plasmatic homocysteine and its relationship to the vitamin status in a developing country. Due to the voluntary participation of the subjects in this protocol, we are well aware of the implicit bias in the design and therefore must consider the reported results as the best possible scenario, ie.: people who is most concerned with his/her health status will be more willing to participate.

The present results support the urgent need to implement folic acid fortification of foods in our country. Besides USA and Canada, such public health policy has been already implemented in Chile and Costa Rica. The values obtained in the present study should serve as the baseline to evaluate the efficacy of future public health policies related to food fortification with folic acid in our country. Recently, it has been reported that use of folic acid, vitamin B6 and B12, in patients that have already had a cardiovascular event (IM) was associated with a poor outcome [28,40]. It should be stressed, however that the dose used was about three times higher than the recommended daily ingest of folic acid and about six times the values for used in food fortification. In any case, these results, which

do not contradict the potential benefits of fortification calls for caution in their use as treatment in such patients. Since fortification has been in effect in the US and Canada for only about ten years, it is still premature to draw conclusions about its potential benefit for the prevention of cardiovascular disease, and, in any case should be taken only as a way of modulating one of the many factors involved in the pathogeny of the disease.

Although atherothrombosis is the leading cause of mortality in Venezuela (~36 % of all causes of death) [32], we can not at the moment establish any relationship with the prevalence of hyperhomocysteinemia in our population. However since data on the epidemiology of cardiovascular disease in Venezuela is available, in the long term, the possible beneficial effects of folic acid fortification may help to elucidate the relationship of hyperhomocysteinemia with cardiovascular disease.

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