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Total plasma homocysteine values among elderly subjects: Findings from the Maracaibo Aging Study

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Abstract

Objectives: The present study generated baseline data for total homocysteine (tHcy) in elderly Caribbeans of Hispanic ancestry, residing in Venezuela, their country of origin.

Design and methods: 2106 participants in the Maracaibo Aging Study (MAS), older than 55 years, underwent standardized clinical and laboratory assessments, including measurement of plasma tHcy levels, folate, and vitamin B12 in fasting samples.

Results: tHcy concentration in the healthy, normative group ranged from 4.1 to 31.8 μmol/L, with a median of 11.5±4.7 μmol/L. tHcy level increased with age, was significantly higher in men than in women, and exhibited inverse correlations with folate and vitamin B12.

Conclusions: tHcy levels of the MAS participants were generally higher than levels previously reported for community-dwelling elderly populations from other countries. The normative centile curves for tHcy can be used in disease risk analysis for this population, and possibly for other Hispanic populations residing in the Caribbean.

Keywords: Aging; Risk factor; Caribbean; Homocysteine; Elderly

Introduction

Homocysteine (Hcy) is an amino acid derived from methionine demethylation. To reconstitute methionine, Hcy can acquire a methyl group from methyltetrahydrofolate in a reaction catalyzed by vitamin B12-dependent methyltransferase [1]. Age and gender affect the plasma levels of Hcy and previous studies have shown that values of plasma total Hcy (tHcy) increase throughout life, and that men exhibit higher values than women [2,3]. Diet, micronutrient supplementation, smoking, and alcohol consumption may further influence tHcy levels [4–7]. Although it has been suggested that the Hispanic populations exhibit lower tHcy levels than non-Hispanic populations [8–11], there is insufficient information on tHcy from different ethnic groups, particularly elderly Hispanics, to test this hypothesis. To date, no tHcy values have been published for elderly Hispanics residing in a developing country. Given differences in nutrition and other lifestyle factors, Hcy levels for these individuals are likely to differ from levels of elderly Hispanics living in developed countries.

High plasma tHcy level constitutes a risk factor for several unfavorable conditions common among elderly people, including cardiovascular diseases [12–17], Alzheimer's disease [18], and colon cancer [19]. To classify an individual as at risk for a specific disease using a laboratory parameter, it is necessary to determine the physiological range of that parameter, and the range for individuals with the disease [20].

Availability of appropriate normative data for elderly populations is critical to overcoming the current uncertainty in

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using tHcy levels to classify an older individual as at risk, and for treatment planning and outcome measurement. Although previous studies measured tHcy levels among elderly free of disease [21–24], no study has provided baseline information about tHcy levels in a Caribbean population of Hispanic descent, residing in their country of origin.

The present work took advantage of an ongoing study of elderly people living in an urban community in Maracaibo, Venezuela, to determine the distribution of tHcy values among subjects older than 55 years. Healthy individuals in this population were used to generate normative tHcy values. The distribution of tHcy values was also determined for a reference sample that exhibited optimal levels of folate and vitamin B12.

Participants and methods

The Maracaibo Aging Study population

Subjects included in this study were participants in the Maracaibo Aging Study (MAS), a population-based, longitudinal study of age-related conditions that took place in Santa Lucia county, near downtown Maracaibo, Venezuela [25]. Santa Lucia contains both traditional houses and recently built, high-rise buildings, and is crossed by important thoroughfares. All available information indicates that the Santa Lucia population does not differ significantly from the rest of the population of Maracaibo or from other urban areas of Zulia State [26]. It has been shown, furthermore, that the population of Zulia does not constitute a genetic isolate separate from the whole population of Venezuela [27]. It has been reported that the population of Maracaibo is derived from an admixture of Europeans and Africans with the local Amerindians[28,29]. Results of previous studies indicated that this population is genetically similar to other populations of Hispanic ancestry in the Caribbean [30,31]. The life-style, diet, and family values of the Maracaibo population, furthermore, are similar to those of other Caribbean Hispanics [32].

Every resident of Santa Lucia older than 55 years of age (3657 individuals) was invited to take part in the MAS. Of the total number eligible to participate, 2438 individuals (66.67%) completed clinical, neuropsychological, cardiovascular, and laboratory assessments between January, 1999 and August, 2000. 908 individuals (26.3%) refused to be assessed; however, the age distribution, education level, and male:female ratio of this group were not significantly different from those of the participants [25], so it is likely that their Hcy levels were also not significantly different. Forty-five individuals (1.3%) did not complete the clinical assessments, and 265 individuals (7.7%) were too ill or died prior to data collection. Ultimately, information concerning plasma tHcy and micronutrients was available for 2106 individuals.

The Ethics Review Board of the Cardiovascular Center (University of Zulia, Maracaibo, Venezuela) approved the MAS. All participants gave informed, written consent, and when doubts about competency of the subject existed, consent from a proxy was also required. Results from individuals who reported food intake during the 12 h fasting period prior to blood donation, or ingestion of alcoholic beverages within 24 h prior to blood collection, or whose blood samples showed signs of hemolysis, were excluded from further analyses.

Groups used to determine normative values and reference intervals of tHcy

The group of MAS participants used to determine normative tHcy values was defined as "healthy" and was based on *a posteriori* selection from the MAS database, according to the recommendations of the International Federation of Clinical Chemistry (IFCC) [33]. Particular effort was made to detect major chronic disorders with systemic impact that could potentially alter the plasma tHcy concentration. The exclusion criteria were as follows: chronic kidney insufficiency (serum creatinine $\geq$2.50 mg/dL in men and $\geq$2.20 mg/dL in women, or medical history), alcoholism, liver cirrhosis, heart failure with history of decompensation, dementia (diagnostic protocol previously reported [25]), gastric or intestinal diseases that could cause malabsorption, liver or pancreatic disorder, cancer, diabetes mellitus with damage to multiple target organs, thyroid disorders, severe chronic obstructive pulmonary disease, severe arthritis, any terminal condition, and current medication with barbituric or cytostatic drugs. Considering that vitamin B12 and folate levels are important determinants of plasma tHcy levels [34,35], and that supplementation of food with micronutrients is not the norm in Venezuela (only 21% of women and 13% of men in the MAS study used vitamin supplements), each individual in the normative group was required to have both vitamin B12 and folate levels above the gender-specific 33rd percentile or first tertile.

Even more stringent criteria were used to select a subset of the normative group as a reference group, in order to determine reference intervals for tHcy: for women, folate $\geq$7 ng/mL, vitamin B12 $\geq$339 pg/mL, and creatinine $\geq$1.01 mg/dL; for men, folate $\geq$6.29 ng/mL, vitamin B12 $\geq$309 pg/mL, and creatinine $\geq$1.24 mg/dL. These values were used by Selhub et al. [36], and are generally considered to be "micronutrient-replete" values, above which the variability of tHcy levels attributable to these micronutrients should be residual [37–39]. In the reference group, 22% of women and 10% of men reported taking vitamin supplements.

Specimen collection and analysis

Blood was collected between 7:00 and 8:00 a.m., after overnight fasting. Samples were taken by venipuncture from an antecubital vein, and collected into EDTA tubes that were immediately placed in crushed ice and protected from light. Within an hour after collection, the plasma was separated by centrifugation at 3000×*g* and 4 °C for 10 min, and immediately stored in aliquots at −70 °C. tHcy, vitamin B12, and folate were estimated using commercial kits for the Abbott IMx analyzer™ (Abbott Park, IL, USA). In these analyses, tHcy is measured by fluorescence polarization immunoassay (FPIA), B12 by microparticle enzyme immunoassay (MEIA), and folate by ion capture assay. The intra-assay variation for tHcy (defined as

the degree of disagreement among three replicates per assay) was 3.5%. For blood samples from 40 individuals analyzed by the MAS, replicate plasma tHcy determinations were made by HPLC at the Instituto Venezolano de Investigaciones Cientificas in Caracas, and at the University of Texas Southwestern Medical School, using a high-performance liquid chromatographic method based on that of Araki and Sako [40]. The values reported by the three independent laboratories were not significantly different (MAS = 12.11 ± 0.6283, Caracas = 12.13 ± 0.6786, Texas = 12.05 ± 0.8748, $p > 0.5$), and the inter-series coefficient of variation was 4.2%. In each case, the standard error of the mean [SEM] of the difference between the measurements by the MAS and the other laboratory was negligible (MAS vs. Caracas = 0.42, MAS vs. Texas = 0.17). The Pearson's correlation coefficient (r^2) between absolute values from MAS analyses and the HPLC results from Caracas and Texas was 0.99 in each case, which is comparable to the precision in other reports using the FPIA method [41].

Statistical analyses

The median, interquartile range (IQR), and the upper 95th and 97.5th percentiles of tHcy levels were calculated for the subject group as a whole and for two groups divided by gender. As tHcy was not normally distributed, but positively skewed, we used the Mann–Whitney *U* test to compare tHcy levels between males and females. tHcy concentrations were log-transformed and tested for normality by inspecting the normal plot and using the Anderson–Darling test. The log of tHcy fulfilled the criteria for and conformed to normality (Anderson–Darling, $p \geq 0.1$). Simple linear regression and Pearson's correlation coefficient were used to determine how log tHcy varied with age. Statistical analyses were done using SPSS software, version 10.0.

Partitioning of the normative and reference samples

As tHcy levels are affected by physiological functions that vary with age and gender, and individuals older than 55 years constitute a broad group, the reference group was divided into six age groups: 55–59, 60–64, 65–69, 70–74, 75–79, and ≥ 80 years. The Harris and Boyd method [42], adapted by Horn and Pesce [43], was used to determine if tHcy levels differed significantly among these subgroups, or whether the samples could be combined in the computation of a reference interval.

Centiles and reference interval computation

Selected centiles for tHcy were calculated for age and gender subgroups of the normative population while reference intervals for tHcy were calculated for gender subgroups of the reference population. tHcy data were log-transformed, and data points >4 SD from the log-transformed mean were discarded. Parametric reference values were calculated from log-transformed data according to methods recommended by the IFCC [33]. Means (±1.96 × SD) were calculated for each group, and the 90% confidence interval was calculated as the mean (±2.81 × SD/n), where n = number of subjects in that group. The parametric data were then back-transformed and presented in the original units.

In addition to parametric analyses, we used a simple nonparametric approach suggested by IFCC [33] and NCCLS [44] guidelines. In summary, values were ordered, and percentiles were obtained as $p/(100(n+1))$, where p = the percentile, and n = sample size. We also applied an alternative computation of percentiles, $p/(100(n+0.5))$, suggested by Lentner [45]. A bootstrap-based, resampling method was used to estimate the 2.5 and 97.5 percentiles [46]. Finally, results of the simple and bootstrap-based procedures were used to determine 90% confidence intervals for the percentiles. CBstat software, version 4.3 (Denmark), was used for computation of percentiles.

Distribution of tHcy values vs. age

The limits of age-specific normative intervals were defined by curves which incorporated changes between the upper and lower percentiles. This was necessary, as the standard deviation varied with age. A semi-parametric approach, applying a nonparametric kernel-smoother, but with normally distributed residuals, was used to estimate the centiles of tHcy values across ages. Because gender-specific levels of tHcy were significantly different, data for male and female subjects were analyzed separately. To ensure normality of the residuals, the data were transformed, and the transformations that gave the best fit were used for gender-specific data. The data were natural-log transformed for male subjects, and square-root transformed for female subjects. For each analysis, a smoothing parameter was derived from the data by cross-validation. Both the cross-validation function and the smoothing function were part of a public-domain package available for the R programming environment [47]. Centiles were fitted assuming normality of the residuals, and using an estimate of the error standard deviation produced from the smoothing function.

Results

Characteristics of the entire MAS population

The age of the 2438 participants of the MAS who underwent full clinical assessment [40] ranged from 55 to 101 years (Table 1). Men constituted 33.2% of the sample, and were slightly, but significantly older than women (67.9 and 66.4 years, respectively; Mann–Whitney *U* test, $p < 0.001$). Health status of the participants was heterogeneous, ranging from healthy, self-sufficient persons to bed-ridden individuals.

Characteristics of the normative and reference groups

The normative group of healthy individuals was selected by review of the overall MAS database. Of the total MAS participants, 1520 were excluded on the basis of health problems or medical history, or because they exhibited folate and vitamin B12 levels in the lowest tertile for their gender. Another 13 individuals were excluded because their tHcy levels were outliers (>3 SD of the group mean for their gender). The

Table 1
Characteristics of elderly men and women in the entire Maracaibo Aging Study (MAS), the healthy, normative group, and the reference group

	All MAS participants	Normative group			Reference group		
		Total	Women	Men	Total	Women	Men
Number	2438	903	601	302	136	92	44
Age [y]							
Mean (±SD)	67.4 (±9.0)[a]	66.5 (±8.3)	66.9 (±8.4)[b]	65.3 (±8.1)	65.94 (±8.3)	67.2 (±8.6)[c]	63.2 (±7.1)
Range	55–101	55–96	55–96	55–92	55–87	55–87	55–83
Education, [y] (±SD)	5.9 (±4.2)	6.1 (±4.3)	5.3 (±4.0)[b]	7.8 (±4.4)	6.6 (±4.5)	5.5 (±3.6)[c]	9.1 (±5.2)
Plasma tHcy [μmol/L]							
Median (±SD)	14.5 (±5.5)[a, d]	11.5 (±4.7)[e]	11.0 (±4.4)[b]	12.6 (±4.9)	10.8 (±3.5)	10.30 (±3.6)[c]	11.4 (±3.0)
Range	3.1–115.9	4.1–31.8	4.1–26.8	6.2–31.8	5.7–26.8	5.7–26.8	8.1–19.6
Creatinine [mg/dL]							
Mean (±SD)	0.92 (±0.46)	0.89 (±0.28)	0.81 (±0.23)[b]	1.03 (±0.29)	0.81 (±0.16)	0.76 (0.15)[c]	0.91 (0.15)
Range	0.10–9.40	0.10–2.50	0.10–2.50	0.40–2.20	0.30–1.10	0.30–1.01	0.4–1.10
Cholesterol [mg/dL]							
Mean (±SD)	192.6 (±58.6)[a]	199.2 (±59.2)	209.2 (±58.7)[b]	180.5 (±55.6)	199.2 (±62.0)	211.6 (±58.1)[c]	173.2 (±62.5)
Range	1.5–676.0	1.6–407.0	74.0–407.0	59.0–374.0	72.0–376.0	74.0–376.0	72.0–371.0
Body mass index (±SD)	27.4 (±5.6)	27.4 (±5.1)[e]	27.6 (±5.2)	27.0 (±5.0)	26.1 (±4.4)	25.9 (±4.1)	26.5 (5.0)
Smoking habit, n (%)							
Never	1220 (50.7)	451 (50.7)[c]	364 (60.6)[b]	94 (31.1)	84 (61.8)	68 (73.9)[c]	16 (36.4)
In the past	808 (36.6)	317 (35.6)	180 (29.9)	141 (46.8)	20 (14.7)	8 (8.7)	12 (27.3)
Current smoker	378 (15.7)	122 (13.7)	57 (9.5)	67 (22.1)	32 (23.5)	16 (17.4)	16 (36.4)

[a] Whole population and reference group are significantly different.
[b] Normative women and men are significantly different.
[c] Reference women and men are significantly different.
[d] From 2106 subjects.
[e] Normative group and reference group are significantly different.

final reference group of healthy elderly subjects comprised 905 individuals.

The normative group was comparable to the total MAS population in age, gender ratio, education, Body Mass Index (BMI), smoking habits, creatinine, and cholesterol levels (Table 1). The normative group had a mean age of 66.5±8.3 years (median=65). Women were significantly older than men, were less educated, smoked less, had lower creatinine, and higher cholesterol levels (Mann–Whitney U tests, each $p<0.01$), but showed no significant difference in BMI. This lack of difference in BMI between genders may be caused by different proportions of men and women older than 80 years (18.5% of men vs. 26.6% of women, Mann–Whitney U test, $p<0.01$).

The reference group did not differ significantly from the normative group in age, gender ratio, education level, creatinine, or cholesterol levels (Table 1). However, the plasma tHcy levels and BMI were significantly lower for the reference group. The reference group also included higher percentages of individuals that never smoked or that were current smokers.

Distribution of total plasma homocysteine

Plasma levels of tHcy, folate, and vitamin B12 were determined for 2106 MAS participants (86.4% of those that completed the clinical assessment). The frequency distribution of plasma tHcy in this population was not Gaussian (Fig. 1), but was positively skewed (skewness=2.1±0.06) and leptokurtic (kurtosis=6.9±0.112). tHcy concentration ranged from 3.23 to 115.90 μmol/L, with a mean of 14.15±7.13 μmol/L, and was significantly higher in men than women (15.6±8.7 vs. 13.4±6.1, respectively; Mann–Whitney U test, $p<0.001$). tHcy level showed a positive correlation with age (Fig. 3; $r^2=0.25$, $p<0.001$). This effect was similar for both genders ($r^2=0.25$ for men and 0.26 for women). Despite this significant correlation, comparisons of mean tHcy level between contiguous age subgroups using the Harris and Boyd method [42] did not show any significant differences. Grouping participants by decades instead of 5-year intervals, furthermore, did not generate contiguous subgroups that were statistically different. These results show that the change in tHcy with age was continuous; there was no particular age at which tHcy level increased stepwise.

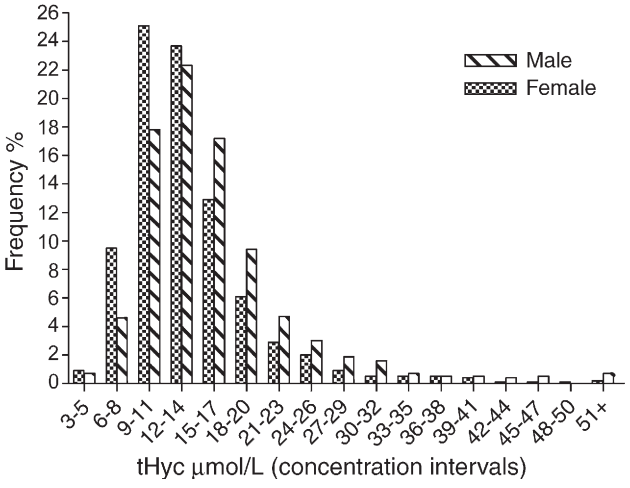

Fig. 1. Frequency distributions of plasma total homocysteine (tHcy) values among the elderly (≥55 years) population of the Maracaibo Aging Study (MAS). Data are shown separately for women (checkered bars) and men (hatched bars).

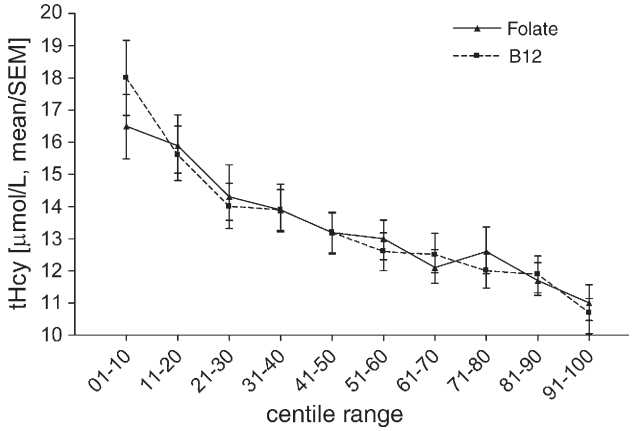

Fig. 2. Mean plasma homocysteine concentrations (±1 SE) corresponding to deciles of vitamin B12 and folate, determined for the entire MAS population.

Plasma tHcy level exhibited a significant inverse relationship with both folate and vitamin B12 (Fig. 2; $r^2=0.068$ for folate and 0.034 for B12, $p<0.0001$). The relationship between plasma tHcy level and folate was consistent across the entire range of folate concentrations; i.e. the magnitude of the difference in tHcy concentrations from the lowest to the highest folate decile was constant. In the case of vitamin B12, tHcy level showed the strongest decline in the lowest three deciles of the vitamin. Even though men exhibited higher plasma tHcy levels than women, the relationships of tHcy with folate and vitamin B12 were similar for the two groups. The overall population averages for folate and vitamin B12 were 5.4± 3.6 ng/mL and 435.7±424 pg/mL, respectively. Tertile values for folate were 3.5 and 5.0 ng/mL for men, and 4.0 and 5.6 ng/mL for women. Tertile values for vitamin B12 were 215 and 342 pg/mL for men, and 261 and 452 pg/mL for women.

Plasma total homocysteine levels in the normative group

Plasma tHcy levels in the normative group were significantly lower than in the general MAS population (Table 1, Mann–Whitney *U* test, $p<0.0001$). tHcy values, furthermore, were significantly higher in men than in women (Mann–Whitney *U* test, $p<0.0001$) through all age groups (Fig. 3). tHcy values for the normative group exhibited a distribution similar to that of the overall MAS population—positively skewed, rather than Gaussian. Log(tHcy) varied significantly with age for both genders ($r^2=0.244$ for men and 0.273 for women, $p<0.0001$).

tHcy values for both men and women in the reference group increased smoothly and consistently with age (Fig. 4). Men had consistently higher values than women, except for subjects ≥90 years of age; in this age group, women had higher tHcy values. Median tHcy concentration for different age groups ranged from 12.1 to 15.9 µmol/L in males, and from 9.8 to 18.1 µmol/L in females. Men exhibited their highest tHcy levels at age 85, after which tHcy concentration decreased slightly. Women, in contrast, did not reach maximum tHcy levels until 93 years of

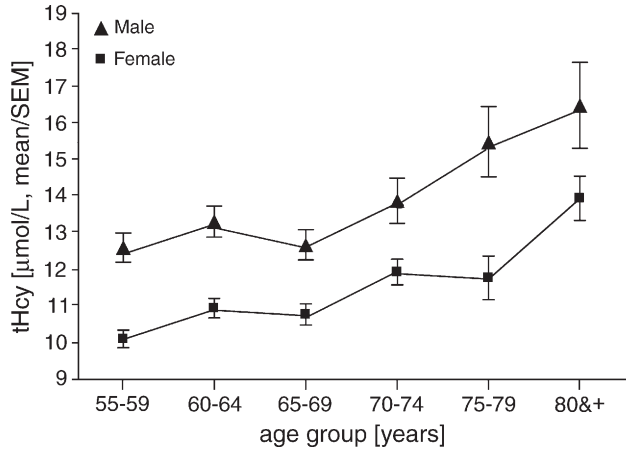

Fig. 3. Mean homocysteine concentrations (±1 SE) in the normative group, divided by age and gender.

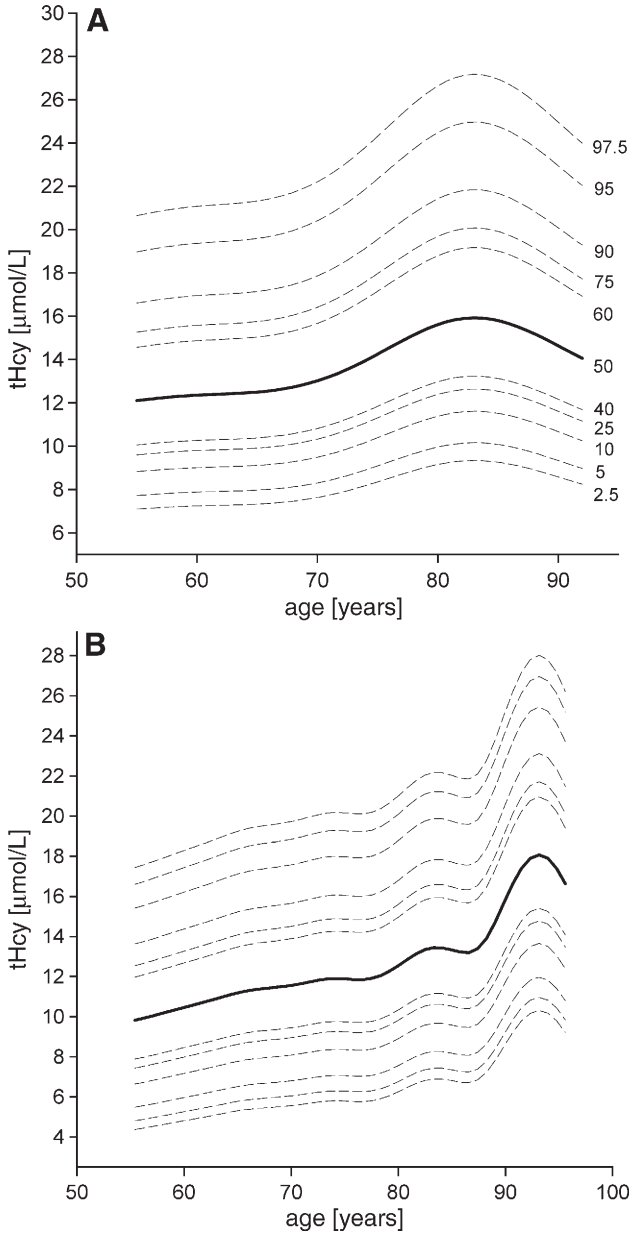

Fig. 4. Median and selected centile values of plasma homocysteine vs. age for (A) men and (B) women in the normative group.

age, with a fairly pronounced increase between 87 and 93 years, and decrease after age 93. The distances from the median to the 5th and 95th centiles were similar across the entire age range, indicating that the variances of the distributions did not change appreciably with age. One drawback of the study, however, was the limited number of subjects in the oldest age group; only 55 women and 19 men were more than 80 years old.

Normative intervals for plasma total homocysteine

Gender-specific centiles of tHcy level were calculated for the normative group using parametric and nonparametric methods (Table 2). The centile values were consistently greater in men than in women. Parametric and nonparametric methods provided similar results, with bootstrapping improving slightly the range between the upper and lower percentiles for the central 95%.

Reference intervals for plasma total homocysteine

The 10th and 90th centiles of tHcy concentration in the reference group were used as reference intervals for each gender (Table 3). We also included the 5th and 95th percentiles to allow comparisons with other studies. The percentile values were consistently greater in men than in women.

Discussion

The present study provides the first report of tHcy values for a population of elderly Hispanic Caribbeans, residing in their native country. The distribution of normative tHcy values presented herein provides baseline data that can be compared to tHcy values of individuals with specific medical disorders or risk levels to evaluate plasma tHcy as a diagnostic tool. The age- and gender-specific distributions can also be used to determine if the plasma tHcy of an apparently healthy individual is within the expected range. A physician can use the centile curves for healthy elderly subjects to "locate" the tHcy level of a new patient of known age and gender. Such nomograms have not been previously developed for tHcy levels in elderly populations.

We believe that the plasma tHcy levels reported herein accurately represent healthy elderly Venezuelans, based on the conservative strategies used in sampling, laboratory analyses, and data analyses. First, we selected a reference population that was examined clinically, limited participants to those at the high end of the folate and micronutrient spectrum, and excluded participants with conditions that specifically affect tHcy concentration. Second, error from laboratory analyses was minimized, as all tests were performed using the same instruments and operators, and analytical results were validated at two other independent laboratories. The precision of these sampling methods was evidenced by the narrow confidence intervals of the higher reference limit (21.6–25.5 μmol/L in men, 19.1–21.8 μmol/L in women). Finally, several different statistical methods were used to estimate reference intervals. The consistency of the reference intervals determined using the parametric and nonparametric (simple and bootstrapping) methods suggested that the reference group represented a single population, and that diseased individuals did not constitute a significant portion of the sample. These rigorous data analyses are necessary to generate reliable reference values for use in disease risk assessment in populations of shared ancestry and similar lifestyle and habits to the population studied.

In accordance with previous studies of elderly populations [4,34,48–50], results of the present study showed that tHcy levels increased with age, independent of gender and health status. Furthermore, our results indicated that this increase occurred gradually, rather than in distinct steps. This suggests that the use of graphs showing percentiles vs. age as continuous curves is more accurate than the use of a single normative value for all ages or values for artificially segregated age groups. There was a decline in tHcy values in men older than 85 years and in women older than 90 years that might be attributable to the low number of healthy subjects in the highest age groups.

Table 2
Percentile values (with 90% confidence intervals) of plasma homocysteine levels [μmol/L] for males and females in the normative group

Males		Percentiles	Females	
Parametric	Nonparametric		Parametric	Nonparametric
22.51 (21.50–23.59)	23.52 (21.77–28.68)[a]	97.5	19.62 (18.96–20.30)	20.46 (18.93–22.20)[b]
20.57 (19.75–21.46)	20.53 (18.90–23.18)	95	17.87 (17.34–18.43)	18.51 (17.08–19.04)
18.54 (17.87–19.24)	18.39 (17.78–19.43)	90	16.06 (15.63–16.49)	15.99 (15.46–16.47)
17.29 (16.71–17.87)	17.36 (16.45–18.00)	85	14.92 (14.57–15.30)	14.90 (14.33–15.31)
16.35 (15.83–16.88)	16.33 (15.77–17.10)	80	14.10 (13.76–14.43)	13.97 (13.66–14.38)
15.58 (15.12–16.05)	15.55 (14.92–16.23)	75	13.41 (13.12–13.71)	13.42 (12.99–13.77)
14.92 (14.50–15.36)	14.85 (14.39–15.41)	70	12.81 (12.56–13.11)	12.80 (12.21–13.12)
14.34 (11.60–11.83)	14.35 (13.68–14.83)	65	12.32 (12.06–12.57)	12.00 (11.74–12.42)
13.81 (13.44–14.20)	13.66 (13.29–14.28)	60	11.83 (11.60–12.09)	11.60 (11.43–11.85)
12.85 (12.50–13.20)	12.520 (12.18–13.25)	50	11.48 (11.23–11.71)	11.02 (10.73–11.26)
8.02 (7.69–8.36)	8.27 (7.80–8.70)	5	6.75 (6.55–6.96)	5.40 (6.48–7.16)
7.33 (6.99–7.67)	7.43 (6.51–8.12)[c]	2.5	6.15 (5.94–6.36)	6.02 (5.73–6.49)[d]

[a] Bootstrap results 23.54 (21.57–25.50).
[b] Bootstrap results 20.45 (19.07–21.84).
[c] Bootstrap results 7.41 (6.62–8.20).
[d] Bootstrap results 6.07 (5.76–6.38).

Table 3
Homocysteine levels [μmol/L] in community dwelling elderly in different countries compared with same age-range group of the entire population of the Maracaibo Aging Study

Reference, country	Age	Females				Males			
		(*N*) Mean±SD				(*N*) Mean±SD			
		Other studies		This study		Other studies		This study	
Nygard, 1995 [4], Norway	65–67	(1932)	11.58±0.2	(376)	13.28±4.19	(1386)	12.93±0.32	(238)	15.57±7.46
Rasmussen, 1996 [51], Denmark	60–69	(18)	10.34±2.74	(448)	13.42±4.39	(17)	9.90±2.13	(292)	15.61±7.61
	≥70	(19)	10.81±3.40	(518)	13.98±6.11	(19)	11.86±2.34	(270)	14.56±6.31
Bates, 1997 [52] Britain	≥65	(373)	14.68±6.3	(726)	13.81±5.78	(375)	15.67±7.43	(402)	14.82±6.99
Stehowver, 1998 [53], The Netherlands	64–84	N/A		(705)	13.84±5.67	(878)	15.8±8.2	(405)	14.99±7.12
Rossi, 1999 [54], Australia	61–70	(116)	12.7±3.7	(438)	13.59±4.67	(125)	13.9±3.4	(305)	15.49±7.61
	>70	(56)	13.5±4.3	(475)	13.98±6.15	(59)	15.3±5.3	(230)	14.41±6.10
Jacques, 1999 [9], USA									
Non–Hispanic Whites	60–69	(191)	10.2±3.8	(448)	13.42±4.39	(206)	11.5±8.3	(292)	15.61±7.61
	70–79	(305)	11.5±6.8	(367)	13.82±5.85	(158)	12.8±8.3	(201)	14.95±6.52
	≥80	(204)	12.4±6.3	(151)	14.35±6.72	(175)	13.4±6.0	(69)	13.39±5.52
Non-Hispanic Blacks	60–69	(111)	11.0±4.9	(448)	13.42±4.39	(120)	12.3±5.2	(292)	15.61±7.61
	70–79	(60)	11.0±4.9	(367)	13.82±5.85	(66)	13.8±5.7	(201)	14.95±6.52
	≥80	(40)	14.9±6.9	(151)	14.35±6.72	(26)	13.4±5.8	(69)	13.39±5.52
Mexican-Americans	60–69	(144)	9.8±3.6	(448)	13.42±4.39	(156)	11.7±3.8	(292)	15.61±7.61
	70–79	(61)	9.9±3.5	(367)	13.82±5.85	(65)	12.0±5.0	(201)	14.95±6.52
	≥80	(29)	11.6±4.0	(151)	14.35±6.72	(29)	17.7±12.7	(69)	13.39±5.52
Bostom, 1999 [34], USA	59–91	(1138)	11.27[a]	(998)	12.59[a]	(795)	12.40[a]	(606)	13.64[a]
Saw, 2001 [50], Singapore	55–64	(98)	9.4±0.5	(522)	13.64±4.78	(86)	11.3±0.7	(373)	15.49±6.95
	65–74	(56)	10.3±0.7	(424)	13.72±5.41	(53)	12.8±1.0	(264)	15.37±7.61
Wolters, 2003 [55], Germany	60–70	(178)	9.88±2.41	(491)	13.46±4.52	N/A		(332)	15.59±7.58

N/A: Not available.
[a] Geometric mean.

The higher mean tHcy concentrations found in men compared to women, in the general MAS population and in the normative and reference groups, were predictable results, as other studies reported similar gender differences [3,5,14]. The difference between genders was evident at all ages, indicating that independent reference ranges should be maintained for men and women in diagnosing hyperhomocysteinemia in elderly individuals.

tHcy levels found in the MAS population were generally higher than levels previously reported for other populations of community-dwelling elderly [9,34,50–56], even when similar age and gender groups are compared (Table 4). To our knowledge, there has been only one other study of tHcy in an elderly Caribbean population of Hispanic origin, and that population resided in the United States [36]. It is of particular interest to compare our results to the tHcy values of that population, using the same selection criteria for vitamin B12, folate, and creatinine levels (Table 3). The central 80% intervals for plasma tHcy of the reference group of MAS participants were 8.6 to 17.3 μmol/L for men and 7.3 to 14.6 μmol/L for women. The upper ends of these reference intervals are much higher than those of the healthy elderly Mexicans residing in the U.S. As our reference group included individuals older than 55 years, while the previous study included only individuals older than 60 years, the higher reference intervals of the MAS population are even more significant. These differences emphasize the danger of extrapolating reference values from populations in developed countries to those in developing countries, even among populations that share the same ethnic background. Differences in diet, lifestyle, and healthcare access among populations in different countries may have differential effects on tHcy level. It would be of interest to replicate our study using other populations of community-dwelling elderly of Hispanic ancestry residing in the Caribbean to ascertain the validity of extrapolation on a regional scale.

As tHcy levels are partly dependent on micronutrients, a deficiency in folate and vitamin B12 could have accounted for the high levels found in the present study. However, the measured concentrations of these micronutrients were similar to those reported by other studies of elderly subjects [13,50,57] where tHcy plasma levels were not as high as those of the MAS. Furthermore, we used values considered

Table 4
Reference intervals by percentile for plasma total homocysteine (tHcy) determined in the present study and by Selhub et al. [36]

	Present study	Selhub et al.
Age group	≥55	≥60
Females		
n	92	185
5% [μmol/L]	6.8	4.9
10% [μmol/L]	7.3	5.3
95% [μmol/L]	18.5	11.6
90% [μmol/L]	14.6	10.3
Males		
n	44	156
5% [μmol/L]	8.4	5.9
10% [μmol/L]	8.6	6.3
95% [μmol/L]	18.5	15.3
90% [μmol/L]	17.3	12.3

Note that both studies used the same vitamin B12, folate, and creatinine levels as criteria for selecting reference groups (see Participants and methods above).

to be "micronutrient-replete" [36,37,51,56,58] to estimate reference values for comparison with other elderly populations [36]. Another potential factor that could have influenced tHcy levels found in the present study is that individuals with high levels of methyl malonic acid were not excluded. High levels of this metabolite, which was not measured, are associated with high levels of plasma tHcy. MTHFR genotypes were also not taken in account. These genotypes have been reported to influence tHcy levels, and this influence may be modulated by both folate and vitamin B12 [6,59].

One potentially important method for controlling the cost of healthcare, especially for the ever-increasing number of elderly individuals, is the appropriate use of diagnostic and therapeutic technology [60]. Unless information on biological variation is available, the usefulness of conventional, population-based values will remain unknown [61]. This is particularly true for understanding changes in metabolites that result from the interaction of age and lifestyle. The present study reports baseline information on plasma tHcy in healthy, elderly Caribbeans by age and gender, which could provide the basis for developing a diagnostic algorithm for specific diseases in elderly patients, particularly cardiovascular or cognitive affectation. The normative values and reference intervals presented herein could also help to monitor response to therapies that treat hyperhomocysteinemia in aged individuals. The applicability of our results to other elderly populations certainly deserves further study.

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