Influence of age, sex, folate and vitamin B_{12} status on plasma homocysteine in Saudis

Mohammed Salleh M. Ardawi, PhD, FRCPath, Abdulrahim A. Rouzi, FRCSC, Mohammed H. Qari, FRCPA, Foaud M. Dahlawi, PhD, Rajaa M. Al-Raddadi, MBBS.

ABSTRACT

Objective: To evaluate the reference intervals for fasting total plasma homocysteine concentrations in Saudi healthy males and females in relation to age, sex and the nutritional status of folate and vitamin B_{12}.

Methods: A prospective study was conducted on randomly selected Saudi healthy males (n=642) and females (n=784) living in the Jeddah area, Kingdom of Saudi Arabia. Plasma homocysteine together with serum folate and plasma vitamin B_{12} concentrations were determined. Analysis of variance was used to examine differences among various groups according to age, sex or folate, or both or vitamin B_{12} status for different variables. Correlations were carried out using multiple linear regression analysis.

Results: Reference intervals for plasma homocysteine concentrations in Saudi healthy males and females (age 20 -69 years) was documented. The age-adjusted geometric mean of plasma homocysteine concentration was significantly greater in males (9.91 umol/L) than in females (8.08 umol/L) ($P<0.0001$). In both males and females, values for serum folate and plasma vitamin B_{12} concentrations significantly and negatively correlated with plasma homocysteine concentrations ($P<0.000$). Serum total cholesterol showed significant positive correlations with plasma homocysteine in both males ($r=0.448$, $P<0.000$) and females ($r=0.313$; $P < 0.000$). Diastolic ($r= 0.182$; $P<0.001$) and systolic ($r=0.309$; $P < 0.000$) blood pressure values showed significant positive correlations with plasma homocysteine concentrations in females only. Stepwise multiple linear regression analysis showed that in both males and females, age, sex, serum folate, and waist-to-hip ratio and plasma vitamin B_{12} were significant determinants of plasma homocysteine concentrations.

Conclusion: The first data on plasma homocysteine concentrations in Saudi healthy males and females are reported. Age and sex differences were confirmed and a significant inverse relationship between plasma homocysteine concentrations and that of serum folate and plasma vitamin B_{12} was observed. Various independent variables including age, sex, serum folate, waist-to-hip ratio and plasma vitamin B_{12} contributed to the changes in plasma homocysteine. Plasma homocysteine concentrations should be evaluated in patients at risk for cardiovascular and other related diseases in the Saudi population.

Keywords: Homocysteine concentrations, folate, vitamin B_{12}, cardiovascular diseases.

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Homocysteine (HCY) is a naturally sulphur-containing amino acid formed during the metabolism of the essential amino acid methionine.[1] Normally HCY is rapidly metabolized either by remethylation to methionine, a process requiring folate and vitamin B_{12} (cobalamin), or by trans-

From the Department of Clinical Biochemistry, (Ardawi), Department of Obstetrics & Gynecology, (Rouzi), Department of Hematology, (Qari), Faculty of Medicine and Allied Sciences and Department of Computer Engineering, (Dahlawi), Faculty of Engineering, King Abdul-Aziz University, Department of Primary Health Care, (Raddadi), Directorate of Health Affairs, Ministry of Health, and the Department of Laboratory Medicine, (Ardawi), New Jeddah Clinic Hospital, Jeddah, *Kingdom of Saudi Arabia*.

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Address correspondence and reprint request to: Professor Mohammed Salleh M. Ardawi, PO Box 20724, Jeddah 21465, *Kingdom of Saudi Arabia*. Tel. +966 (2) 6922705. Fax. +966 (2) 6403203. E-mail: ardawims@yahoo.com

sulphuration pathway that involves a vitamin B6–dependent enzymatic reaction forming cystathionine[2] **(Figure 1)**.[3]

In 1975, McCully and Wilson[4] proposed the "homocysteine theory of arteriosclerosis" on the basis of pathological examinations of autopsy material from homocysteinuric children.[5] During the last 10 years, HCY has emerged among other risk factors such as cholesterol, smoking and obesity, as a major independent risk factor for cardiovascular, cerebrovascular and peripheral vascular diseases.[6-13] Indeed, an increment of 5 umol/L in the total fasting plasma HCY concentrations was shown to be associated with a 60-80% higher risk of coronary artery disease, a 50% higher risk of cerebrovascular disease and a 6-fold higher risk of peripheral vascular disease.[6,14]

The determinants of total plasma HCY concentrations are complex and include demographic (age, sex, ethnicity),[15-19] genetic (namely mutations at the levels of enzymes),[16, 20-22] and acquired factors.[14,16] The latter include both the state-of-health and life-style (namely exercise, smoking habits, coffee consumption) considerations.[14] Moreover, fasting hyperhomocysteinemia was found to be associated with lower circulating levels and intakes of folate and vitamin B_{12}[11,15] which was also amenable to therapy with these vitamins.[23-26]

There is little available information describing plasma HCY concentration in healthy Saudi males and females. In the present study, reference intervals for fasting total plasma HCY (thereafter referred to as plasma HCY) levels were measured in a sample of the local Saudi healthy population living in the Jeddah area, Kingdom of Saudi Arabia (KSA) in relation to age, sex and the nutritional status of folate and vitamin B_{12}. The results are discussed and compared with other studies carried out on other populations.

Methods. A total of 1426 healthy Saudi males (n=642) and females (n 784) living in Jeddah, KSA participated in the present study. Subjects were randomly selected during a nutritional health survey from 14 primary health care centers scattered around the city of Jeddah, KSA. Subjects who agreed to participate in the survey were asked to visit a special clinic at King Abdul-Aziz University Hospital (KAUH), Jeddah, KSA to be enrolled in the present

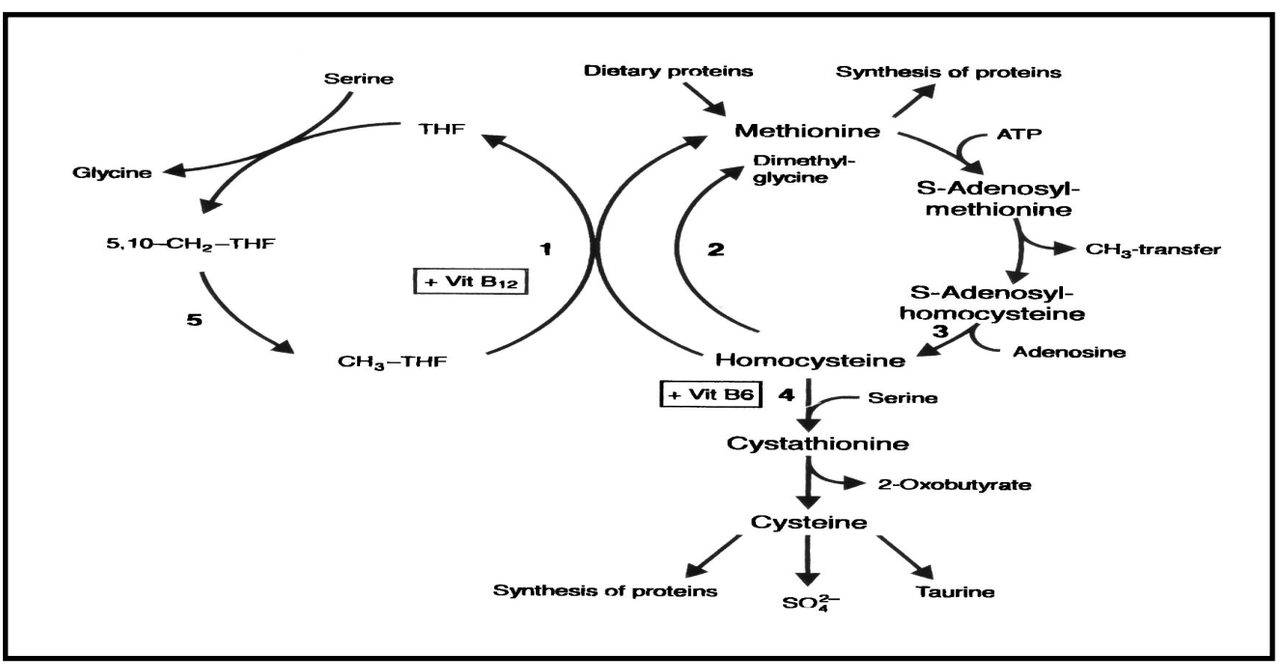

Figure 1 - The metabolism of homocysteine. The enzyme (1) 5-methyltetrahydrofolate: homocysteine methyltransferase (EC 2.1.1.13), which uses cobalamin (vitamin B_{12}) as a coenyzme, transfers a metyl group from 5 - methyltetrahydrofolate (CH_3-THF) to homocysteine to form methionine. 5-methyltetrahydrofolate is made by reduction of 5, 10-methylenetetraydrofolate (5, 10-CH_2-THF), the compound of central importance in folate metabolism, by the enzyme (5) 5, 10-methylenetetrahydrofolate reductase (EC 1.7.99.5). An alternative pathway for the methylation of homocysteine to methionine is mediated by the enzyme (2) betaine: homocysteine methyltransferase (EC 2.1.1.5) using betaine as methyl donor. S-Adenosylmethionine is the methyl donor in a wide range of transmethylation reactions. The loss of the methyl group results in the formation of S-adenosylhomocysteine, which is subsequently converted to homocysteine by the enzyme (3) S-adenosylhomocysteine hydrolase (EC 3.3.1.1). In the trans-sulphuration pathway, homocysteine is condensed with serine to form cystathionine by the pyridoxal phosphate (vitamin B_6) - dependent enzyme (4) cystathionine β-synthase (EC 4.2.1.22) [Adapted from Ref 3]. ATP -adenosine triphosphate, SO_4^2 - sulphate anions.

Table 1 - Anthropometric and blood pressure measurements in Saudi subjects studied according to age and sex.

Measurements	Sex	20-29 years	30-39 years	40-49 years	50-59 years	60-69 years
n	Male	138	191	182	85	46
	Female	136	256	245	96	51
Age (years)	Male	24.76 ± 2.72	34.41 ± 3.23	45.85 ± 2.84	54.61 ± 3.11	65.44 ± 2.93
	Female	24.71 ± 2.89	36.05 ± 3.56	45.66 ± 2.92	56.29 ± 3.03	65.79 ± 2.86
Body mass index (kg/m^2)	Male	24.23 ± 1.81	27.90 ± 4.04	26.62 ± 2.92	28.14 ± 5.80	26.49 ± 4.02
	Female	21.55 ± 2.32	29.18 ± 5.93	29.47 ± 6.01	29.02 ± 5.10	30.13 ± 4.84
Waist-to-Hip ratio	Male	0.81 ± 0.07	0.94 ± 0.07	0.93 ± 0.05	0.97 ± 0.05	0.98 ± 0.08
	Female	0.79 ± 0.06	0.81 ± 0.09	0.85 ± 0.11	0.88 ± 0.09	0.93 ± 0.10
Diastolic blood pressure (mm Hg)	Male	76 ± 6	75 ± 7	79 ± 7	82 ± 13	80 ± 9
	Female	73 ± 8	72 ± 10	76 ± 11	82 ± 11	79 ± 8
Systolic blood pressure (mm Hg)	Male	110 ± 12	119 ± 15	125 ± 13	131 ± 20	128 ± 17
	Female	105 ± 16	115 ± 17	122 ± 18	136 ± 15	135 ± 19

Data presented as mean ± SD for 642 males and 784 females, n-number

study. Age, body weight, height, body mass index (BMI) (kg/m^2), and waist-to-hip ratio (WHR) were recorded. Blood pressure was determined with a mercury manometer while participants were in a sitting position after being allowed 15 minutes rest. Age and anthropometric data of the subjects studied are presented in **Table 1**. Each subject was medically examined and interviewed using a standardized questionnaire to collect information on life style, smoking habits, level of physical activity in leisure time; coffee and tea consumption and the use of vitamins and medications. Subjects with renal, hepatic, gastrointestinal or with evident cardiovascular or endocrine disorders or on any form of drug treatment were excluded. Subjects, who are cigarettes or sheesha smokers or are on vitamin supplement(s) were also excluded from the present study. In addition, all subjects included exhibited: 1. Normal blood counts; 2. Normal values for renal creatinine (serum creatinine in females <105 umol/L and males <116 umol/L,); and 3. Normal values for liver function tests (serum aspartate aminotransferase (AST) <30 U/L; alanine aminotransferase (ALT) <30 U/L; alkaline phosphatase (ALP) between 80-280 U/L; and gamma-glutamyl transferase (GGT) <60 U/L). Fasting blood samples (10-12 hours overnight) were collected in the morning between 09:00-11:00 hours for the measurements of the various analytes studied. The study protocol was in agreement with KAUH, Jeddah, KSA ethical standards and the Helsinki Declaration of 1975, as revised in 1989.

Ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood for the analysis of HCY and vitamin B_{12} was immediately centrifuged (3000xg for 10 minutes at 4ºC) for the preparation of plasma. Plasma was stored at -130ºC until analysis. Serum was used for the measurements of folate and other biochemical parameters including creatinine, AST, ALT, ALP, GGT, uric acid, total cholesterol and triglycerides.

Plasma HCY was measured by an enzymatic immunoassay based on a fluorescence polarization immunoassay technique using the IMX System (Abbott Laboratories, Abbott Park, Illinois 60064, United States of America, (USA)) with dedicated reagents obtained from Abbott Laboratories, USA. It employs the patented Axis enzymatic conversion of HCY to s-adenosyl-L-homocysteine (SAH). The 3 step assay includes 1. Reduction (with dithiothreitol) and enzymatic conversion (SAH-hydrolase) to produce SAH; 2. Addition of the anti-SAH antibody; and 3. Addition of the fluorescein tracer.[27] Plasma vitamin B_{12} and serum folate were measured by a competitive binding assay technique based on an electrochemillunescence immunoassay using the Elecsys 2010 System (Boehringer Mannheim GmbH, D-68298 Mannheim, Germany) with dedicated reagents obtained from Roche Diagnostic GmbH, D-68298, Mannheim, Germany. Serum total cholesterol and other biochemical parameters studied were measured by commercially available kits using a Hitachi 912 Autoanalyzer (Roche Diagnostics GmbH, D-68298 Mannheim, Germany) with dedicated reagents obtained from Roche Diagnostics, Germany.

Statistical analysis. Results are presented as means (±SD). Data were analyzed using statistical package for social sciences (SPSS) (version 11.0 for Windows Smart Viewer) supplied by SPSS Inc. 2000, Mapinfo Corp. Tokyo, New York, USA. Results that were not normally distributed were log-transformed before analysis. Analysis of variance

Table 2 - Geometric means, untransformed means and selected percentiles of fasting plasma total homocysteine concentrations by age group and sex in Saudi subjects studied.

Age (years)	n	Geometric mean	Mean	SD	Selected percentiles umol/L				
		umol/L			5th	25th	50th	75th	95th
Males									
20-29	138	8.37[a]	8.57[a]	1.80	5.60	6.80	6.90	10.20	11.19
30-39	191	8.18[b]	8.43[b]	2.18	5.90	6.85	7.65	9.95	13.30
40-49	182	10.55[a]	10.68[a]	2.06	7.92	9.28	10.40	12.26	13.50
50-59	85	11.64[a]	11.75[a]	1.55	8.80	10.68	11.85	12.91	14.06
60-69	46	15.69[a]	15.78[a]	1.65	13.08	14.14	15.89	17.09	18.45
All	642	9.91[a]	10.31[a]	2.90	6.10	8.10	10.29	12.30	16.15
Females									
20-29	136	6.34	6.56	1.84	4.50	5.30	6.00	7.40	10.48
30-39	256	7.31	7.68	2.49	4.49	5.40	7.20	9.50	12.50
40-49	245	8.74	9.01	2.38	5.08	7.53	8.70	10.28	13.60
50-59	96	10.25	10.50	2.12	5.78	9.55	10.70	11.65	13.95
60-69	51	12.73	12.86	1.19	10.18	11.22	12.30	14.19	17.03
All	784	8.08	8.51	2.71	4.60	6.30	8.20	10.41	13.52

a - significantly different (ANOVA) from corresponding age group in female subjects (P<0.000)
b - significantly different (ANOVA) from corresponding age group in female subjects (P<0.021), n- number, SD - standard deviation

was used to examine differences among the groups for different variables, and the Bonferroni criterion was used when significance tests were made. Correlations were carried out using multiple linear regression analysis.

Results. Reference intervals for plasma HCY were determined for 1426 Saudi healthy subjects (males 642 and females 784) included in the study. The distribution of subjects by age group, sex, geometric means, untransformed means and selected percentiles of plasma HCY are presented in **Table 2** and is also shown in **Figure 2**. The age-specific plasma HCY concentrations were lower in females than in males for each age group. The age adjusted geometric mean of plasma HCY concentrations was significantly greater in males (being 9.91 umol/L) than in females (being 8.08 umol/L) (P<0.0001). A significant age-sex interaction (P<0.001) was observed indicating that the relationship between age and plasma HCY concentrations differed between males and females (**Table 2**). Age-specific means of plasma HCY concentrations tend to increase across all age categories. The rate of increase was rapid in both males and females across age groups except in males aged 20-39 years (**Table 2**).

In both males and females, values for serum folate and plasma vitamin B_{12} were significantly and negatively correlated with plasma HCY concentrations (**Figures 3 & 4**), and correlations remained significant after exclusion of subjects with subnormal serum folate (<4.0 nmol/L) and plasma vitamin B_{12} (<130 pmol/L) values (**Tables 3 & 4**). Serum total cholesterol showed significant positive correlations with plasma HCY in both males (r= 0.448; P<0.000) and females (r=0.313; P<0.000). Diastolic (r=0.182; P<0.001) and systolic (r=0.309; P<0.000) blood pressure values showed significant positive correlations with plasma HCY concentrations in females only, but not in males included in the present study (**Table 4**). To examine further the relationship between plasma HCY concentration and other variables that may influence its concentration, stepwise multiple linear regression analysis was carried out on males and females studied (**Table 5**). In males, age, serum folate, systolic blood pressure and plasma vitamin B_{12} significantly contributed to the variation in plasma HCY values with minor contributions from BMI, WHR, serum total cholesterol, serum uric acid and diastolic blood pressure. In females, age, serum folate and plasma vitamin B_{12} significantly contributed to the variation in plasma HCY values with no significant contributions from other variables examined. When stepwise multiple linear regression analysis was carried out on all pooled Saudi males and females studied with plasma HCY as a dependent variable retained age, sex, serum folate, WHR, and plasma vitamin B_{12}; as important determinants, (**Table 5**).

Discussion. The present study is the first report on reference intervals on plasma HCY concentrations in Saudi males and females of various age groups (20-69 years). The results showed that plasma HCY concentrations increased across all age groups examined (with the exception of males aged 20-39

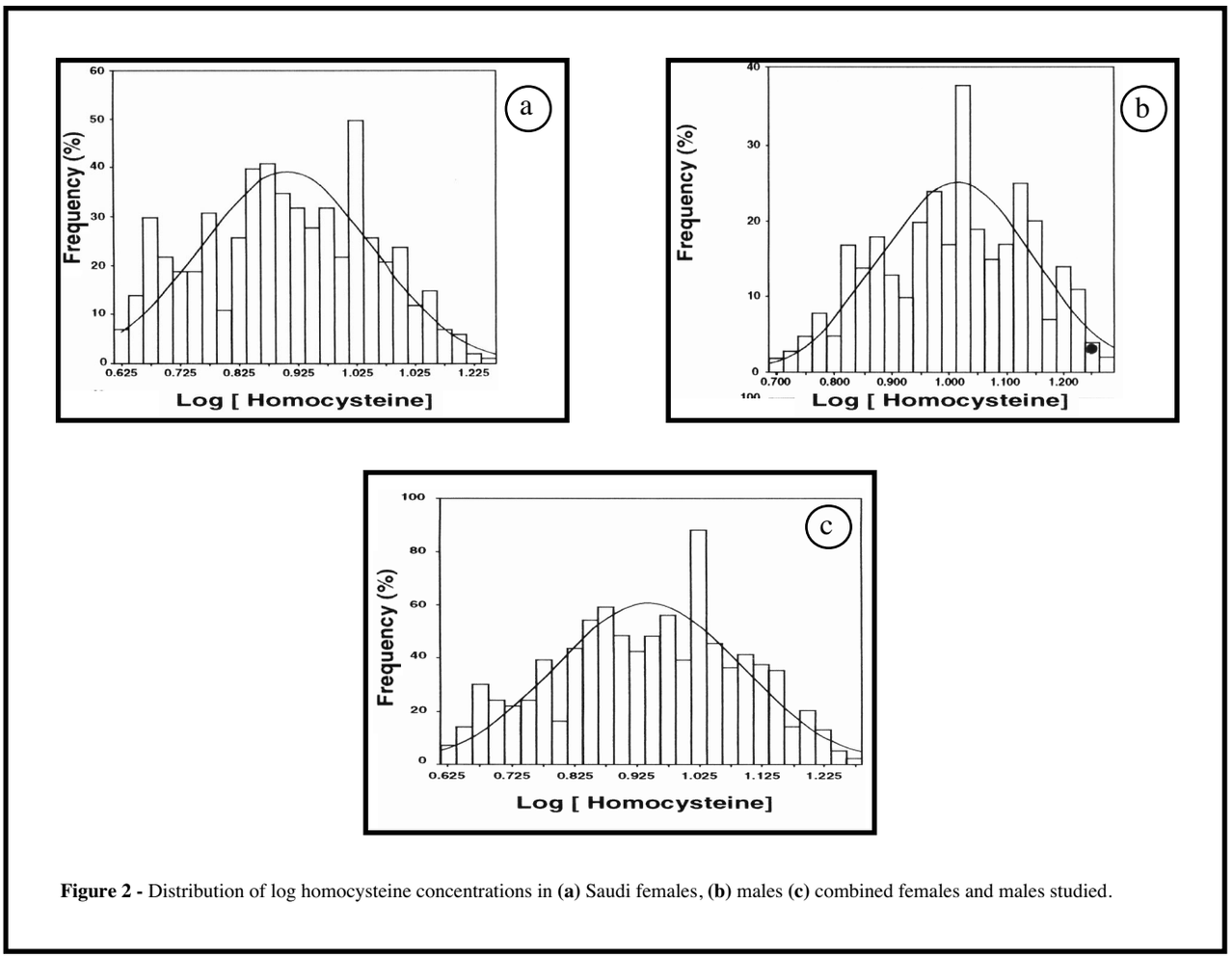

Figure 2 - Distribution of log homocysteine concentrations in **(a)** Saudi females, **(b)** males **(c)** combined females and males studied.

years) and were higher in males than in females of all age groups: The mean of plasma HCY of all males showed a 21.2% increase over that of all females (**Table 2**). The reference intervals or "normal values" for plasma HCY differ somewhat from one study to another, but values of plasma HCY between 5 umol/L and 15 umol/L are usually considered normal.[28] The variability may be related to several factors

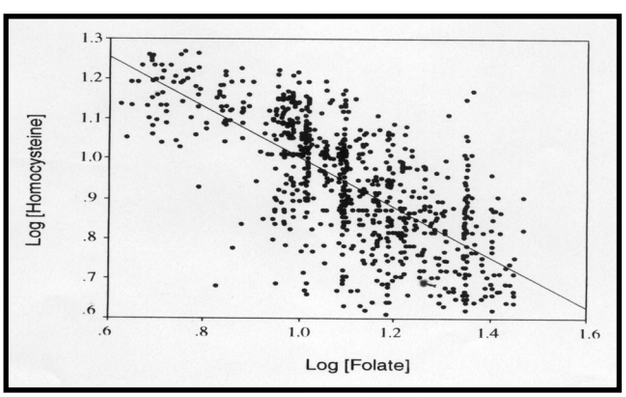

Figure 3 - The relationship between log folate concentration and log homocysteine concentration in Saudi females and males studied.

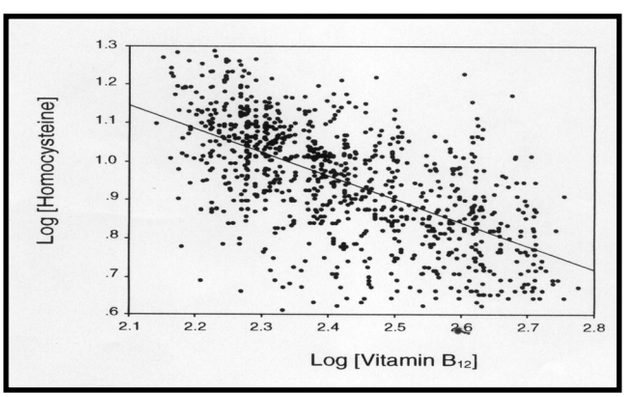

Figure 4 - The relationship between log vitamin B_{12} concentration and log homcysteine concentration in Saudi females and males studied.

Table 3 - Concentrations of fasting plasma total homocysteine, serum folate and plasma vitamin B12 in males and females studied.

Age (Years)	Sex	n	Concentration of		
			Plasma homocysteine (umol/L)	Serum folate (nmol/L)	Plasma vitamin B12 (pmol/L)
20-69	Male Female	642 784	10.31 ± 2.90[a] 8.51 ±. 2.71	12.27 ± 4.84[a] 14.40 ± 5.26	239 ± 73[b] 292 ± 102
Significantly different (ANOVA) from corresponding female subjects: a (P<0.000); b (P<0.001), n- number					

including: Different methods used for the measurement of plasma HCY;[29] differences in the sample(s) processing;[29] or the selection of the studied subjects[29] who are influenced by various factors (namely, ethnicity, genetic and life-style) that may contribute to the variation in the concentrations of plasma HCY.[30-31] In the present study, the upper limit for plasma HCY (mean +2 SD for all males and females) is within the low risk values described by others,[29-31] although only 27 (1.9%) subjects (22 males and 4 females aged 55-69 years) exhibited values >15 umol/L with the highest value being 18.20 umol/L. A total of 492 (34.5%) subjects exhibited values for plasma HCY >10.0 umol/L; the latter is considered to be the desirable cut-off value for plasma HCY. Graham et al,[7] found that in men and women younger than 60 years, the risk for cardiovascular disease started to rise from the middle distribution of plasma HCY (being 10.3 umol/L). In a study comparing survivors of myocardial infarction and non-coronary subjects,[31] the referent level of plasma HCY was 9.8 umol/L. Moreover, the referent level for the risk of death associated with plasma HCY was <9.0 umol/L[8] or <10.0 umol/L.[32] However, it has been shown that the risk for coronary artery disease is represented by a continuum of plasma HCY concentration, with substantial risk occurring between 10 umol/L and 15 umol/L.[28,33,34] Indeed, the American Heart Association have arbitrarily defined hyperhomocysteinemia as being divided into moderate, intermediate and severe, referring to plasma HCY concentrations being <15-30, 31-100 and >100 umol/L.[28] Subjects with coronary artery, cerebrovascular and peripheral vascular diseases usually present with mild hyperhomocysteinemia (15 umol/L - 25 umol/L).[4-8,33,34]

The age and sex related differences in plasma HCY are consistent with other studies of adult males and females.[15-16,35] In healthy American males and females, as part of the National Health and Nutrition

Table 4 - Spearmans correlation (r-values) and their significances (P-values) between fasting plasma homocysteine and other variables in subjects studied.

Variables	Males (n=642)		Females (n=784)		All (n=1426)	
	r - value	p - value	r - value	p - value	r - value	p - value
Age (years)	0.677	0.000	0.588	0.000	0.602	0.000
BMI (kg/m^2)	0.11	0.867	0.138	0.003	0.005	0.891
Waist-to-hip ratio	0.029	0.660	0.041	0.371	0.030	0.435
Diastolic blood pressure (mm Hg)	0.071	0.420	0.182	0.001	0.181	0.000
Systolic blood pressure (mm Hg)	0.065	0.459	0.309	0.000	0.245	0.000
Serum folate	-0.736	0.000	-0.622	0.000	-0.661	0.000
Plasma vitamin B12	-0.665	0.000	-0.561	0.000	-0.597	0.000
Serum cholesterol	0.448	0.000	0.313	0.000	0.311	0.000
Serum uric acid	-0.044	0.516	0.045	0.338	0.167	0.000
n - number, BMI - body mass index						

Table 5 - Regression equation of the variables that were found to influence fasting plasma total homocysteine concentrations in Saudi males and females studied.

Independant variables	Regression statistics			
	β	SE (β)	t-value	p-value
Age (years)	0.118	0.006	20.63	0.000
Serum folate (nmol/L)	–0.361	0.027	-13.61	0.000
Waist-to-hip ratio	2.284	0.647	3.533	0.000
Plasma vitamin B_{12} (pmol/L)	0.004	0.001	2.826	0.005
R^2 - 0.689, SE - standard error β - coefficient regression				

Examination Survey, a total of 3,766 males and 4,819 females aged 20-80 years were examined: the age-adjusted means of plasma HCY concentrations in non-Hispanic white males (being 9.6 umol/L) was 21.5% higher than in the corresponding females (being 7.9 umol/L).[18] In the Hordaland (Norway) Homocysteine Study, a total of healthy 7,591 males and healthy 8,585 females aged 40-67 years were evaluated: In the young group (40-42 years), plasma HCY concentrations in the males (being 10.8 umol/L) were 19% higher than in the females (being 9.1 umol/L).[16] This difference was decreased to 11% in the older age group examined (65-67 years) being 12.3 and 11 umol/L in males and females; the latter represented a 13% increase in males and a 21% increase in females over the younger age groups.[16] Brattstrom et al[17] studied 131 males and 113 females aged 35-95 years. They observed higher levels of plasma HCY in males in all age groups with females exhibiting an increase beyond the age of ≥ 65 years. Koehler et al[36] evaluated plasma HCY concentrations in males (n=50) and females (n=50) aged 68-96 years and found that males exhibited plasma HCY values 15% higher than that observed in females and concentrations increased significantly with age. Clarke et al,[37] in subjects aged 65-74 years, showed that plasma HCY was ~ one umol/L higher in males than in females and higher in older (>70 years) than in younger (<70 years) individuals. However, no significant age-related differences were observed in a study of 584 healthy Canadian adults: but this sample was fairly young (mean age: 36 years; range: 23-59 years).[38]

Changes in renal function (namely declining of glomerular filtration rate with age);[11] impaired renal metabolism of HCY[39] or vitamin status, or both[28-30] may be responsible in part for the higher plasma HCY concentrations at older age. The sex difference in plasma HCY may be related to menopausal status,[40] or vitamin status, or both.[41] Menopause has been implicated as a determinant of plasma HCY concentrations,[40,42] but little direct evidence exists to relate estrogen concentrations to HCY metabolism. vander Mooren et al,[43] showed that estrogen replacement therapy markedly decreased plasma HCY concentrations in postmenopausal females. In addition, Wouters et al,[42] showed that plasma HCY concentrations were inversely related to estradiol concentrations in pre-menopausal females.[43] Furthermore, the sex difference in plasma HCY may also be explained by differences in body mass, as creatinine synthesis is higher in males than in females. Accordingly, in healthy subjects, a significant correlation exists between plasma HCY and plasma creatinine concentrations.[17] Thus, a part from a possible increase in creatinine levels at older age that results from impaired renal function; this may indicate enhanced HCY production as a consequence of methyl group transfer during creatinine metabolism, and since creatinine production is related to body mass, circulating creatinine might explain in part the sex-related difference in the concentrations of plasma HCY.[16] Indeed, metabolic studies have shown that, in males, each HCY molecule is converted to methionine on average 1.9 times, whereas, in females, the recycling rate is 1.5 times for each molecule.[44]

Folate and vitamin B_{12} nutritional status is another important determinant of plasma HCY concentrations, as illustrated by various studies.[14,19,34,37,45] In the present study, there was a significant inverse relationship between plasma HCY and the concentrations of serum folate and plasma vitamin B_{12}. These observations are consistent with previous studies.[15,25,46-48] In the present study, only 84 (5.9%) subjects exhibited serum folate concentrations between 4.22-6.36 nmol/L, however, none of the subjects exhibited serum folate ≤ 4.0 nmol/L; a cut-off level to indicate folate deficiency.[49] These subjects were aged between 60-69 years, of them only 15 (1.06%) exhibited plasma HCY >15 umol/L (being 16.35 ± 0.94 umol/L; mean ± SD). Plasma/serum HCY is known to markedly increase in folate-deficient patients.[45,50-51] In a study by Kang et al,[50] evaluating the relationship between homocysteinemia and folate deficiency the following observations were obtained in a group of 200 patients. Nineteen patients exhibiting subnormal levels of serum folate (<4.0 nmol/L); 137 subjects exhibiting low-normal levels (4-7.8 nmol/L), and 44 subjects exhibiting normal levels (8-35.8 nmol/L), serum HCY was negatively correlated with serum folate concentration. In 84% of the subjects with subnormal serum folate, HCY was 2 SD greater than the normal mean, and reaching a value up to 70 umol/L. Of particular interest was the finding that more than 5% of the subjects studied by Kang et al[50] exhibiting low-normal serum folate showed increased serum HCY concentrations. The possibility that subjects with no clinical or laboratory

indication of folate deficiency may actually be deficient in intracellular folate is supported by a decrease in plasma HCY after folate supplementation.[41] The effect of folate was observed both in hyperhomocysteinemic postmenopausal females and in apparently, healthy subjects.[15,41] Indeed, in the present study, the change in serum folate concentrations was one of the independent variables that significantly contributed to the changes in plasma HCY (**Table 5**), however, but none of the subjects studied in the present work was considered to be folate-deficient.

Concentrations of HCY in plasma/serum are increased in most patients with vitamin B_{12} deficiency.[51-53] In a large population of patients with vitamin B_{12} deficiency, serum vitamin B_{12} and plasma HCY concentrations displayed a hyperbolic relationship.[29] The plasma HCY levels increased abruptly when serum vitamin B_{12} approached values that were below normal (<130 pmol/L), but a negative correlation was observed in the normal to low normal range of vitamin B_{12} values (namely 130-300 pmol/L). Indeed, none of the subjects examined in the present study showed plasma vitamin B_{12} concentrations <130 pmol/L. Based on linear multiple regression analysis, changes in plasma vitamin B_{12} contributed significantly to the variations of plasma HCY ($P<0.005$) (**Table 5**). Therefore, although plasma HCY reference intervals studied in Saudi males and females were found to vary significantly between sexes; this may reflect differences in the expression of the enzymes involved in folate and vitamin B_{12} metabolism as well as, metabolic differences in the extent of methylation and trans-sulphuration pathways.

In conclusion, the present work shows that: (a) reference intervals for plasma HCY were established for Saudi males and females aged 20-69 years; (b) plasma HCY concentrations increased with increasing age and were significantly higher in males than in females; (c) a total of 493 (34.8%) subjects exhibited values for plasma HCY >10 umol/L with only 27 (1.9%) subjects with values >15 umol/L; (d) a significant inverse relationship between plasma HCY concentrations and that of serum folate and plasma vitamin B_{12} levels was observed; and (e) various independent variables including age, sex, serum folate, WHR and plasma vitamin B_{12} contributed to the changes in plasma HCY. Our understanding and appreciation of the factors that influence circulating HCY levels are still not all clear. Since mild increases in plasma HCY concentrations are strongly associated with a greater risk of cardiovascular disease,[4-13] it is very important to evaluate the levels of plasma HCY in subjects who are at risk for cardiovascular or other related diseases, or both in relation to age, sex and other factors that may influence variations in plasma HCY concentrations. Indeed, the American Heart Association[28] recommends that a high-risk strategy approach should be considered: this may include screening for fasting plasma HCY associated with increased risk status; plasma HCY being ≥10 umol/L, in selected patients with personal or family history of premature cardiovascular disease, as well as in patients with malnutrition, malabsorption syndromes, hypothyroidism, renal failure, or systemic lupus erythematosus. In addition, patients undergoing the use of some therapeutic agents including: nicotinic acid, theophylline, bile acid-binding resins, methotrexate, and L-dopa or with recent nitrous-oxide exposure are also included. These intriguing series of associations should enforce intervention studies on the effect of HCY-lowering therapy and influence the design and analysis of future studies on plasma HCY concentrations in subjects at risk for cardiovascular disease.

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