Influence of age, sex, folate and vitamin B₁₂ status on plasma homocysteine in Saudis

Mohammed Salleh M. Ardawi, PhD, FRCPath, Abdulrahim A. Rouzi, FRCS, Mohammed H. Qari, FRCPA, Foad 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 B₁₂.

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 B₁₂ concentrations were determined. Analysis of variance was used to examine differences among various groups according to age, sex or folate, or both or vitamin B₁₂ 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 B₁₂ 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 B₁₂ 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 B₁₂ was observed. Various independent variables including age, sex, serum folate, waist-to-hip ratio and plasma vitamin B₁₂ 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 B₁₂, cardiovascular diseases.

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sulphuration pathway that involves a vitamin B6-dependent enzymatic reaction forming cystathionine (Figure 1). In 1975, McCully and Wilson proposed the "homocysteine theory of arteriosclerosis" on the basis of pathological examinations of autopsy material from homocysteinuric children. 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. 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.

The determinants of total plasma HCY concentrations are complex and include demographic (age, sex, ethnicity), genetic (namely mutations at the levels of enzymes) and acquired factors. The latter include both the state-of-health and lifestyle (namely exercise, smoking habits, coffee consumption) considerations. Moreover, fasting hyperhomocysteinemia was found to be associated with lower circulating levels and intakes of folate and vitamin B12 which was also amenable to therapy with these vitamins.

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 B12. 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 study.

Figure 1 - The metabolism of homocysteine. The enzyme (1) 5-methyltetrahydrofolate: homocysteine methyltransferase (EC 2.1.1.13), which uses cobalamin (vitamin B12) as a coenzyme, transfers a methyl group from 5 - methyltetrahydrofolate (CH3-THF) to homocysteine to form methionine. 5-methyltetrahydrofolate is made by reduction of 5, 10-methylenetetrahydrofolate (5, 10-CH2-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 B6) - dependent enzyme (4) cystathionine β-synthase (EC 4.2.1.22) [Adapted from Ref 3]. ATP -adenosine triphosphate, SO42- - sulphate anions.
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Table 1 - Anthropometric and blood pressure measurements in Saudi subjects studied according to age and sex.

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Sex</th>
<th>20-29 years</th>
<th>30-39 years</th>
<th>40-49 years</th>
<th>50-59 years</th>
<th>60-69 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Male</td>
<td>138</td>
<td>191</td>
<td>182</td>
<td>85</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>136</td>
<td>256</td>
<td>245</td>
<td>96</td>
<td>51</td>
</tr>
<tr>
<td>Age (years)</td>
<td>Male</td>
<td>24.76 ± 2.72</td>
<td>34.41 ± 3.23</td>
<td>45.85 ± 2.84</td>
<td>54.61 ± 3.11</td>
<td>65.44 ± 2.93</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>24.71 ± 2.89</td>
<td>36.05 ± 3.56</td>
<td>45.66 ± 2.92</td>
<td>56.29 ± 3.03</td>
<td>65.79 ± 2.86</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>Male</td>
<td>24.23 ± 1.81</td>
<td>27.90 ± 4.04</td>
<td>26.62 ± 2.92</td>
<td>28.14 ± 5.80</td>
<td>26.49 ± 4.02</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>21.55 ± 2.32</td>
<td>29.18 ± 5.93</td>
<td>29.47 ± 6.01</td>
<td>29.02 ± 5.10</td>
<td>30.13 ± 4.84</td>
</tr>
<tr>
<td>Waist-to-Hip ratio</td>
<td>Male</td>
<td>0.81 ± 0.07</td>
<td>0.94 ± 0.07</td>
<td>0.93 ± 0.05</td>
<td>0.97 ± 0.05</td>
<td>0.98 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.79 ± 0.06</td>
<td>0.81 ± 0.09</td>
<td>0.85 ± 0.11</td>
<td>0.88 ± 0.09</td>
<td>0.93 ± 0.10</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>Male</td>
<td>76 ± 6</td>
<td>75 ± 7</td>
<td>79 ± 7</td>
<td>82 ± 13</td>
<td>80 ± 9</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>73 ± 8</td>
<td>72 ± 10</td>
<td>76 ± 11</td>
<td>82 ± 11</td>
<td>79 ± 8</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>Male</td>
<td>110 ± 12</td>
<td>119 ± 15</td>
<td>125 ± 13</td>
<td>131 ± 20</td>
<td>128 ± 17</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>105 ± 16</td>
<td>115 ± 17</td>
<td>122 ± 18</td>
<td>136 ± 15</td>
<td>135 ± 19</td>
</tr>
</tbody>
</table>

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

study. Age, body weight, height, body mass index (BMI) (kg/m²), 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 cigarette 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 B12 was immediately centrifuged (3000xg for 10 minutes at 4°C) for the preparation of plasma. Plasma was stored at -130°C until analysis. Serum was used for the measurements of folate and other biochemical parameters including creatinine, AST, ALT, ALP, GOT, 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 B12 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
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 B12 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 B12 (<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 all pooled Saudi males and females studied (Table 5). In males, age, serum folate, systolic blood pressure and plasma vitamin B12 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 B12 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 B12; 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
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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 2 - Distribution of log homocysteine concentrations in (a) Saudi females, (b) males (c) combined females and males studied.

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 B12 concentration and log homocysteine concentration in Saudi females and males studied.
including: Different methods used for the measurement of plasma HCY;\textsuperscript{29} differences in the sample(s) processing;\textsuperscript{29} or the selection of the studied subjects\textsuperscript{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.\textsuperscript{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,\textsuperscript{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,\textsuperscript{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,\textsuperscript{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\textsuperscript{a} or <10.0 umol/L.\textsuperscript{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.\textsuperscript{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.\textsuperscript{28} Subjects with coronary artery, cerebrovascular and peripheral vascular diseases usually present with mild hyperhomocysteinemia (15 umol/L - 25 umol/L).\textsuperscript{4-8,33,34} The age and sex related differences in plasma HCY are consistent with other studies of adult males with and non-coronary subjects.\textsuperscript{31} In healthy American males and females,\textsuperscript{15-16,35} 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,\textsuperscript{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,\textsuperscript{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,\textsuperscript{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\textsuperscript{a} or <10.0 umol/L.\textsuperscript{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.\textsuperscript{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.\textsuperscript{28} Subjects with coronary artery, cerebrovascular and peripheral vascular diseases usually present with mild hyperhomocysteinemia (15 umol/L - 25 umol/L).\textsuperscript{4-8,33,34} The age and sex related differences in plasma HCY are consistent with other studies of adult males and females,\textsuperscript{15-16,35} In healthy American males and females, as part of the National Health and Nutrition

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

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Sex</th>
<th>n</th>
<th>Concentration of Plasma homocysteine (umol/L)</th>
<th>Serum folate (nmol/L)</th>
<th>Plasma vitamin B12 (pmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-69</td>
<td>Male</td>
<td>642</td>
<td>10.31 ± 2.90\textsuperscript{a}</td>
<td>12.27 ± 4.84\textsuperscript{a}</td>
<td>239 ± 73\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>784</td>
<td>8.51 ± 2.71</td>
<td>14.40 ± 5.26</td>
<td>292 ± 102</td>
</tr>
</tbody>
</table>

Significantly different (ANOVA) from corresponding female subjects: a (P<0.000); b (P<0.001), n- number

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

<table>
<thead>
<tr>
<th>Variables</th>
<th>Males (n=642)</th>
<th>Females (n=784)</th>
<th>All (n=1426)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r - value</td>
<td>p - value</td>
<td>r - value</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.677</td>
<td>0.000</td>
<td>0.588</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>0.11</td>
<td>0.867</td>
<td>0.138</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.029</td>
<td>0.660</td>
<td>0.041</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>0.071</td>
<td>0.420</td>
<td>0.182</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>0.065</td>
<td>0.459</td>
<td>0.309</td>
</tr>
<tr>
<td>Serum folate</td>
<td>-0.736</td>
<td>0.000</td>
<td>-0.622</td>
</tr>
<tr>
<td>Plasma vitamin B12</td>
<td>-0.665</td>
<td>0.000</td>
<td>-0.561</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>0.448</td>
<td>0.000</td>
<td>0.313</td>
</tr>
<tr>
<td>Serum uric acid</td>
<td>-0.044</td>
<td>0.516</td>
<td>0.045</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Regression statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \beta )</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.118</td>
</tr>
<tr>
<td>Serum folate (nmol/L)</td>
<td>-0.361</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>2.284</td>
</tr>
<tr>
<td>Plasma vitamin B12 (pmol/L)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

R\(^2\) = 0.689, SE = standard error
\( \beta \) = 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).\(^{16}\) 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 \( \geq 65 \) years. Koehler et al\(^{16}\) 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.\(^{17}\) in subjects aged 65-74 years, showed that plasma HCY was \( \sim \) one umol/L higher in males than in females and higher in older (\( \geq 70 \) 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).\(^{18}\)

Changes in renal function (namely declining of glomerular filtration rate with age):\(^{19}\) impaired renal metabolism of HCY\(^{19}\) or vitamin status, or both\(^{20-30}\) 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,\(^{31}\) 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.\(^{34}\) 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 (4.7-7.8 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. The effect of folate was observed both in hyperhomocysteinemic postmenopausal females and in apparently healthy subjects. 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 B12 deficiency. In a large population of patients with vitamin B12 deficiency, serum vitamin B12 and plasma HCY concentrations displayed a hyperbolic relationship. The plasma HCY levels increased abruptly when serum vitamin B12 approached values that were below normal (<130 pmol/L), but a negative correlation was observed in the normal to low normal range of vitamin B12 values (namely 130-300 pmol/L). Indeed, none of the subjects examined in the present study showed plasma vitamin B12 concentrations <130 pmol/L. Based on linear multiple regression analysis, changes in plasma vitamin B12 contributed significantly to the variations in 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 B12 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 B12 levels was observed; and (e) various independent variables including age, sex, serum folate, WHR and plasma vitamin B12 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, 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 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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Riyadh Armed Forces Hospital
Department of Neurosciences

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The Neurosciences Department at the Riyadh Armed Forces Hospital (RAFH) advertises two (2) unsponsored positions for physicians to join the Neurology Training Programme for the Board of the Saudi Council for Health Specialties. The training programme is for four (4) years. The first year consists of rotations in internal medicine. The following three (3) years consist of rotations in General Adult Neurology, Neurophysiology, Psychiatry, Pediatric Neurology, Neuroradiology, Neuropathology and other related specialties.

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