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Gender differences in homocysteine concentrations, a population-based cross-sectional study

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Abstract *Background and aims:* High concentrations of homocysteine are considered a risk factor for atherosclerosis and coronary artery disease. The aim of this study was to assess whether or not there are gender differences in the plasma concentrations of homocysteine.
Methods and results: Data were collected from medical records of individuals examined at a screening center in Israel between the years 2000–2014. Cross sectional analysis was carried out on 9237 men and 4353 women. Mean (SD) age of the study sample was 48.4 (9.7) and 47.7 (9.7) years for men and women respectively. Average homocysteine concentrations were 12.6 (5.9) and 9.6 (3.2) μmol/L in men and women respectively ($p < 0.001$). Prevalence of homocysteine concentrations above 15 μmol/L was found to be significantly higher in men than in women; 15.5% vs 3.9% respectively ($p < 0.001$). Low concentrations of vitamin (B12 < 200 pmol/L) and low concentrations of folate (<12 nmol/L) were found to be significantly higher in men than in women 20.4% vs. 16.0% and 18.5% vs. 10.8% respectively. Compared to women, men had a significantly higher odds ratio (95% CI) of having homocysteine concentrations above 15 μmol/L: non adjusted model, 4.47 (3.80–5.26); adjusted model for age, smoking status, body mass index, diabetes mellitus, kidney function and low serum concentrations of vitamin B12 and folate, 3.44 (2.89–4.09).
Conclusion: Plasma homocysteine concentrations are higher in men than in women. This may be a contributing factor to gender differences for developing atherosclerosis and coronary artery disease.

Introduction

It is well recognized that there are gender differences for the common risk factors for atherosclerosis and coronary artery disease. For example a recent report of the American Heart Association, regarding statistics of heart disease and stroke in a USA population, revealed that for a non-Hispanic white population the prevalence of smoking, hypertension, diabetes mellitus (DM), LDL >130 mg/dl and HDL <40 mg/dl were: 17.8% vs. 16.8%; 34.5% vs. 32.3%; 8% vs. 7.4%; 29.3% vs. 32.1% and 28.4% vs. 10.3% for men and women respectively [1].

Since the early 1960s, elevated homocysteine concentrations have been shown to be related to the risk of atherosclerosis [2,3]. In following years numerous studies identified a link between high concentrations of homocysteine and atherosclerosis and coronary artery disease

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[4–8]. Possible mechanisms for the association between homocysteine and atherosclerosis include stimulation of smooth muscle cell growth, reduction in endothelial cell growth, reduction in endothelial cell relaxation and decreased synthesis of high density lipoprotein [9].

Despite the potential link between elevated homocysteine concentrations and atherosclerosis and coronary artery disease, the results of treatment with drugs that lower homocysteine concentrations have been controversial. In the HOPE-2 trial, 5522 subjects received either supplementation treatment with folic acid, vitamin B6 and vitamin B12 or a placebo. The mean homocysteine concentrations decreased in the supplement group however the mean five-year follow-up showed that treatment with supplements to have no effect on the primary combined endpoints of cardiovascular death, myocardial infarction, or stroke [10]. The results of treatment with percutaneous coronary intervention (PCI) are also contradictory. In one randomized study, 553 patients undergoing PCI received either supplemental treatment with folic acid, vitamin B12, vitamin B6 or placebo. In the supplement group there was a decreased incidence of major adverse events after an average follow-up of 11 months**.** Moreover**,** the risk for a composite end points (death, nonfatal myocardial infarction need for repeat revascularization) was also lower in that group [11]. In contrast, in another randomized trial, 636 patients following stent placement received either supplementation treatment or placebo. Yet patients in the treatment group had higher rates of restenosis and a higher percentage required target-vessel revascularization [12]. In summary, despite convincing evidence associating hyperhomocsyteinemia with atherosclerosis and coronary artery disease, clinical trials attempting to lower high homocysteine plasma concentrations in those patients have not shown a conclusive positive effect.

Gender differences in homocysteine concentrations have been described for the last 30 years, however the number of studies is limited [13–16]. They were also carried out on a relative small population. It is recognized that homocysteine levels may be affected by age [14], smoking status [17], body mass index (BMI) [18], diabetes mellitus [19,20], kidney function [21] and by serum concentrations of vitamin B12 and folate [22]. Yet previous studies assessing the effect of gender on homocysteine concentrations have not always adjusted for these confounders.

Therefore, we aimed to assess the gender effect on homocysteine concentrations in a cross sectional study involving a very large cohort of subjects, adjusting for all confounders which can affect the homocysteine concentrations. In addition, since homocysteine concentrations may be affected by estrogen [23,24], we carried out a subgroup analysis comparing subjects younger and older than 55 years, assuming women beyond 55 years to be in menopause.

Methods

Study population

The study population consisted of a cross-sectional sample of men and (non-pregnant) women aged 20–80 years referred by their employers for routine medical screening at a tertiary medical center in Israel between the years 2000 and 2014. None of the subjects was hospitalized at the time. Screening consisted of a thorough medical history and a complete physical examination along with a broad series of blood and urine tests, a chest X ray, an electrocardiogram, an exercise stress test, a respiratory function test, and a full ophthalmology examination. For the purpose of this study, we used the data from each subject's most recent visit.

Data on smoking habits were collected from direct questioning on the day of examination at the screening center. Subjects receiving vitamin B12 or folate supplements were excluded from the analysis.

The various blood tests were performed after an overnight 12-h fast. Analysis of plasma homocysteine concentrations was performed on an Abbott Axsym system. This assay is based on the Fluorescence Polarization Immuno-Assay (FPIA) technology. Bound homocysteine (oxidized form) is reduced to free homocysteine that is enzymatically converted to S-adenosyl-L-Homocysteine (SAH). SAH and labeled Fluorescein Tracer compete for the sites on the monoclonal antibody molecules. The intensity of the polarized fluorescence measured by FPIA optical assembly is proportional to the concentration of homocysteine in the sample.

The normal range of homocysteine concentrations for most laboratories is between 5 and 15 μmol/L [25]. Homocysteine concentrations above 15 μmol/L are considered to be elevated.

A computer program was created to transfer all data, from each visit, into a spreadsheet Excel file. Statistical analysis was performed on this file.

The study was approved by the Helsinki Ethics Committee of Rabin Medical Center.

Statistical methods

Baseline characteristics were compared between men and women using Student's t-test and chi-square test for continuous and categorical variables respectively. The odds ratios (ORs) and 95% CI of having hyperhomocysteinemia in relation to gender were assessed by using a logistic regression method. Univariate analyses were performed in Model 1. Model 2 was adjusted for age alone and Model 3 was adjusted for age, smoking status, BMI, diabetes mellitus, kidney function and low serum concentrations of vitamin B12 and folate. As gender differences are at least partially affected by hormonal changes, all analyses were repeated stratifying two age groups: one before and the other after the age of 55 (by which age menopause is assumed for the female population).

For all analyses a p value of <0.05 was considered significant. All analyses were performed using SAS v. 9.4.

Results

The cross sectional analysis included 13,590 subjects, 32% were women. The clinical and laboratory characteristics of these participants are presented in Table 1.

Table 1 Patients' characteristics by gender.

	Men (N = 9237)	Women (N = 4353)	P value[a]
Age (years), mean (SD)	48.4 (9.7)	47.7 (9.7)	<0.001
BMI (kg/m^2), mean (SD)	27.5 (4.0)	25.7 (4.9)	<0.001
Smokers (%)	16.0	17.0	0.171
Waist circumference (cm), mean (SD)	93.5 (11.1)	79.5 (11.2)	<0.001
Systolic BP (mmHg), mean (SD)	122.2 (13.9)	113.3 (14.8)	<0.001
Diastolic BP (mmHg), mean (SD)	79.1 (7.7)	74.4 (8.4)	<0.001
Hypertension (%)	13.4	6.6	<0.001
eGFR (CKD-EPI) ml/min/1.73 m^2, mean (SD)	95.0 (13.9)	99.7 (13.8)	<0.001
Serum glucose concentration (mg/dl), mean (SD)	100.6 (20.2)	94.2 (15.0)	<0.001
Impaired fasting glucose (%)	17.2	9.1	<0.001
Diabetes mellitus (%)	4.9	3.0	<0.001
Total cholesterol (mg/dl), mean (SD)	196.3 (36.6)	199.3 (37.7)	<0.001
Triglycerides (mg/dl), mean (SD)	138.3 (88.7)	106.1 (61.4)	<0.001
LDL cholesterol (mg/dl), mean (SD)	121.4 (31.2)	117.3 (31.9)	<0.001
HDL cholesterol (mg/dl), mean (SD)	47.5 (10.5)	60.3 (13.6)	<0.001
Hypertriglyceridemia (%)	22.3	10.3	<0.001
HDL cholesterol <40 mg/dl (%)	21.3	3.5	<0.001
Plasma homocysteine concentrations (μmol/L) mean (SD)	12.6 (5.9)	9.6 (3.1)	<0.001
Serum vitamin B12 concentrations (pmol/L) mean (SD)	295 (125)	321 (143)	<0.001
Serum folate concentrations (nmol/L) mean (SD)	19.3 (8.6)	22.5 (10.3)	<0.001

[a] Significance was tested using student t test for continuous variables and chi square test for categorical variables.

Average plasma homocysteine concentrations were found to be 12.6 (5.9) and 9.6 (3.1) μmol/L in men and women respectively ($p < 0.001$). Prevalence of hyperhomocysteinemia was significantly higher in men compared to women i.e. 15.5% % vs 3.9% respectively ($p < 0.001$). Average vitamin B12 serum concentrations as well as average serum folate concentrations were significantly lower in men ($p < 0.001$). Low concentrations of serum vitamin B12 (<200 pmol/L) and low serum concentrations of folate (<12 nmol/L) were significantly higher in men compared to women ($p < 0.001$). Compared to women, men had a significant higher odds ratio (95% CI) for hyperhomocysteinemia: non adjusted model, 4.47 (3.80–5.26); adjusted model for age, 4.44 (3.77–5.23) and adjusted model for age, smoking status, BMI, diabetes mellitus, kidney function and low serum concentrations of vitamin B12 and folate, 3.44 (2.89–4.09).

The subgroup analyses results can be seen in Table 2 and Table 3. These results suggest that estrogen has some effect on homocysteine concentrations as women above the age of 55 had higher concentrations of homocysteine compared to those in the younger group. However, after multi variate analysis for age, smoking status, BMI, diabetes mellitus, kidney function and low serum concentrations of vitamin B12 and folate, men over 55 years still had an OR of 2.67 (1.92–3.71) for hyperhomocysteinemia suggesting male gender per se to be a risk factor for hyperhomocysteinemia.

Discussion

Elevated homocysteine concentrations have been shown to be related to the risk of atherosclerosis and coronary artery disease [2,3], and numerous studies have found an association between high homocysteine concentrations and atherosclerosis and coronary artery disease [4–7]. Various possible mechanisms for this association have been suggested. These include: stimulation of smooth muscle cell growth, reduction in endothelial cell growth and endothelial cell relaxation; and a decreased synthesis of high density lipoprotein [9]. However, there is little data to suggest to the role of gender in homocysteine concentrations and metabolism [13–16].

In this large cohort of 13,590 subjects, we clearly show a significant impact of gender on to the average concentrations of homocysteine, as well as the percentage of those with hyperhomocysteinemia, all in favor of women. In fact,

Table 2 Mean homocysteine serum levels and percentage of subjects with homocysteine serum levels above 15 μmol/L in the different age groups.

		Men	Women	*P* value[a]
Age ≤ 55 y (M = 7124) (W = 3432)	Homocysteine (μmol/L) mean (SD)	12.6 (6.3)	9.4 (3.1)	<0.001
	Hyperhomocysteinemia (%)	15.1	3.4	<0.001
Age > 55 y (M = 2113) (W = 921)	Homocysteine (μmol/L) mean (SD)	12.6 (4.5)	10.5 (3.2)	<0.001
	Hyperhomocysteinemia (%)	16.6	5.8	<0.001

Hyperhomocysteinemia = Homocysteine serum levels >15 μmol/L.
M = number of men. W = number of women.
[a] Significance was tested using student t test for continuous variables and chi square test for categorical variables.

Table 3 Logistic regression models of gender as a determining factor for hyperhomocysteinemia. A cross - sectional study of 13590 adults who underwent screening at Rabin Medical Center, 2000–2014. Odds ratios for men in comparison to women, with adjustments and 95% confidence intervals.

Hyperhomocysteinemia	All subjects (N = 13,590)	Age ≤ 55 y (N = 10,556)	Age > 55 y (N = 3034)
Unadjusted	4.47 (3.80–5.26)	4.99 (4.11–6.07)	3.26 (2.42–4.40)
Model 1	4.44 (3.77–5.23)	4.98 (4.10–6.06)	3.25 (2.41–4.39)
Model 2	3.44 (2.89–4.09)	3.85 (3.13–4.72)	2.67 (1.92–3.71)

Hyperhomocysteinemia = homocysteine serum levels >15 μmol/L.
Model 1 = adjusted for age.
Model 2 = adjusted for age, smoking status, BMI, diabetes mellitus, kidney function and low serum concentrations of vitamin B12 and folate.

these gender differences persisted in a subgroup analysis of the subjects above the age of 55 years, thus eliminating the possibility of the beneficial influence of estrogen on homocysteine concentrations [23,24].

Moreover, it is well known that homocysteine concentrations are affected by concentrations of vitamin B12 and folate, such that low concentrations of these vitamins increase the level of homocysteine [22]. Indeed, in this study, we found significantly lower serum concentrations of these vitamins in men. Data on gender difference in vitamin B12 have recently been published by our group [26]. Taking into account the effect of gender differences in vitamin concentrations, we conducted a multi variate analysis to eliminate the effect of low concentrations of these vitamins. This highlights gender per se, as a possible unique independent factor in the concentrations of homocysteine.

The differences between men and women as to homocysteine concentrations may be explained by gender differences in homocysteine metabolism. Homocysteine is normally metabolized by one of two divergent pathways: *trans-sulfuration* and *methylation*. In the *trans-sulfuration* pathway, homocysteine is converted to cysteine by cystathionine-β-synthase and by cystathionine −γ- lyase where vitamin B6 is a cofactor. In the *methylation* pathway homocysteine is re-methylated to methionine by either methionine synthase -where vitamin B12 and folate are cofactors - or by betaine-homocysteine methlytransferase (BHMT). Methionine is trans-methylated back to homocysteine through the production of S-adenosyl methionine (SAM) and SAH (Fig. 1).

It has been suggested that women have greater flux of homocysteine through the trans-sulfuration pathway and thus lower concentrations of homocysteine [20]. In comparison men have a greater need for creatine synthesis as they have greater muscle mass. The two amino acids involved in the biosynthesis of creatine are glycine and L-arginine. In the first step of biosynthesis these two amino acids are combined by the enzyme arginine glycine amino transferase (AGAT, EC 2.1.4.1) to form guanidinoacetate. The latter is methylated by the enzyme guandinoacetate methytransferase (GAMT, EC 2.1.1.2) to produce creatine. The methyl donor in the latter process is SAM transforming to SAH (Fig. 2). A shown back in Fig. 1, SAH is the precursor of homocysteine [27].

Therefore, higher homocysteine concentrations in men may well be explained both by their need for higher concentrations of creatine and their lower flux of homocysteine through the trans-sulfuration pathway.

The main strength of this study is the inclusion of a large cohort (13,590 men and women) with documented homocysteine concentrations together with complete datasets of clinical and laboratory findings. To put this sample size in perspective the largest epidemiological study assessing homocysteine and estrogen status had a sample size of 8585 subjects [23]. Furthermore, compared to previous studies we adjusted our data to all known factors affecting homocysteine concentrations; including age [14], smoking status [17] BMI [18], diabetes mellitus [19,20], kidney function [21] and vitamin concentrations [22] to show that gender per se is a risk factor for elevated concentration of homocysteine. In contrast to a previous study [23] we also show that gender differences persisted even after menopause which eliminates the beneficial effect of estrogen.

Nevertheless, our study has its limitations. The study group was not drawn from a population sample but from those attending an examination center. This limits the generalizability of the findings. In addition, the cross-sectional design precludes conclusions regarding causality. Lastly, hyperhomocysteinemia may be the result of a genetic variability of the MTHFR enzymatic activity and may be affected by vitamin B6 status. These two factors were not assessed in this study.

In conclusion, gender appears to be a significant factor affecting homocysteine concentrations to the advantage of

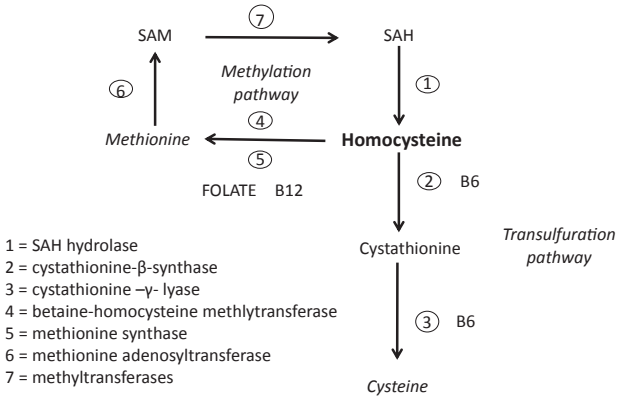

Figure 1 Pathways of homocysteine metabolism. SAH = S-adenosyl homocysteine; SAM = S-adenosyl methionine.

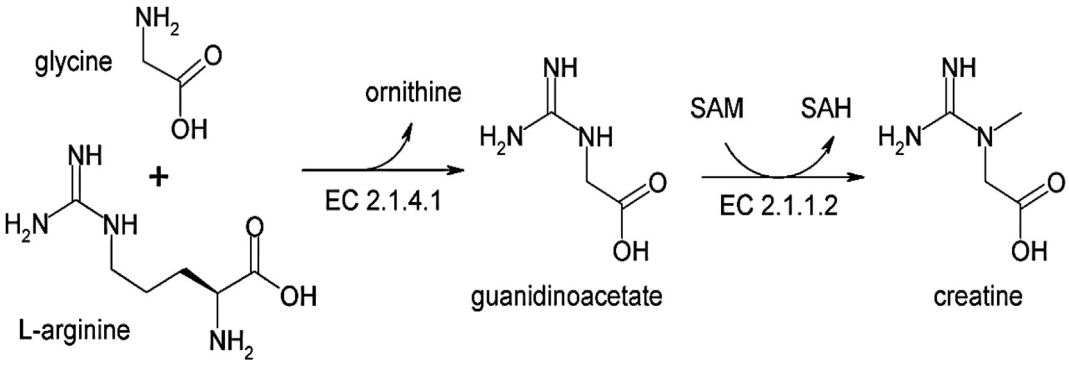

Figure 2 Biosynthesis of creatine EC 2.1.4.1 = arginine glycine amino transferase; EC 2.1.1.2 = guandinoacetate methytransferase.

women. These differences, between men and women, were also shown to exist a in a subgroup of subjects older than 55 years, abolishing the potential impact of estrogen on the homocysteine concentration data. Higher plasma concentrations of homocysteine in men may be another contributing factor to the discrepancy between men and women with regard to the risk of developing atherosclerosis and coronary artery disease.

Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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