Endogenous sex steroids and circulating homocysteine in healthy Greek postmenopausal women. 
Sex steroids and serum homocysteine

G.E. Christodoulakos¹, I.V. Lambrinoudaki¹, D.A. Rizos², A. Alexandrou³, A.V. Kountouris¹, G.C. Creatsas¹

¹²nd Department of Obstetrics and Gynecology, University of Athens, Aretaieion Hospital, ²Hormonal and Biochemical Laboratory, University of Athens, Aretaieion Hospital, Athens, Greece, ³²nd Department of Surgery, University of Athens, Laikon Hospital, Athens, Greece

ABSTRACT
OBJECTIVE: The determinants of serum homocysteine in healthy postmenopausal women remain uncertain. The aim of this study was the assessment of the association of endogenous sex steroids with serum homocysteine levels in healthy postmenopausal women not on hormone therapy. DESIGN: 484 postmenopausal women aged 43-69 years were studied in a cross-sectional design. Parameters assessed were serum FSH, estradiol, total testosterone, SHBG, Free Androgen Index (FAI), Δ4-Androstendione (Δ4A), Dehydroepiandrosterone sulphate (DHEAS) and homocysteine (Hcy). RESULTS: Serum FSH correlated positively (r=0.23, p=0.01), while serum estradiol correlated negatively (r=-0.25, p=0.03) with circulating Hcy. This association remained statistically significant after adjustment for age, years since menopause and BMI. Serum estradiol decreased, while FSH increased linearly with increasing homocysteine quartiles (p=0.04 and p=0.02, respectively). None of the serum androgens assessed correlated with circulating homocysteine. CONCLUSIONS: Endogenous estrogens and not androgens are related to serum homocysteine values in postmenopausal women. Whether this association has clinical implications remains to be clarified.

Key words: Estradiol, Homocysteine, Postmenopausal, Testosterone

INTRODUCTION
The incidence of cardiovascular disease (CVD) rises after menopause and becomes the principle cause of mortality among postmenopausal women.¹ Observational studies have suggested that hormone therapy (HT) administered for the relief of climac-

Abbreviations:
teric symptoms and urogenital atrophy may be cardioprotective.23 Possible mechanisms of cardioprotection include favorable modulation of lipids and lipoproteins, coagulation-fibrinolysis glucose metabolism, promotion of vasodilation, as well as maintenance of vascular endothelial integrity and function and vascular extracellular matrix homeostasis. However, randomized controlled trials (RCTs) have not confirmed the cardioprotective properties of HT.11,12

Homocysteine (Hcy) is a sulfur-containing amino acid and constitutes a risk factor for vascular damage. Hcy has been associated with CVD as well as with recurrent arterial-venous thromboembolism.13 Homozygous or heterozygous defects in genes encoding the enzymes involved in the remethylation (methylene tetrahydrofolate reductase) or transulfuration (cystathionine β-synthase) metabolic pathways as well as deficiencies in nutrients or micronutrients (folate, vitamins B12 and B6) will disrupt Hcy metabolism and increase circulating Hcy levels. Additionally, Hcy levels may be affected by demographic, lifestyle and various health factors.16

Sex steroids appear to influence Hcy metabolism. Hcy levels are lower in women than in men of comparable age, and in premenopausal compared to postmenopausal women. Although Hcy levels increase significantly after menopause, age cannot be excluded as a confounding factor. High Hcy levels among postmenopausal women may be associated with an estrogen-depletion mediated effect or with an age-related evolution of Hcy metabolism. Androgens may also influence Hcy metabolism. Androgens have been reported to increase Hcy levels. This is of particular importance when HT is considered, especially when the co-administered progestin has an androgenic effect and may modify estrogen’s effect on Hcy. This assumption may be reinforced by the results of the estrogen-only arm of the Women’s Health Initiative trial (WHI), which have not indicated an increased risk of estrogen therapy on CVD risk.23

In the aftermath of the HERS and WHI trials, researchers and clinicians aim to elucidate further the effect of HT on CVD risk. We considered that it would be of interest to assess the association of Hcy levels with the endogenous estrogen and androgen levels in healthy non-treated postmenopausal women.

**METHODS**

**Subjects**

Four hundred eighty-four postmenopausal women aged 43-69 years were included in the study. Subjects were recruited from the Menopause Clinic of the 2nd Department of Obstetrics and Gynecology, University of Athens, Aretaieion Hospital. The participants were at least 2 years postmenopausal. All women had not received HT for at least 6 months. None of the participants was under vitamin B supplements.

All patients underwent a gynecological and biochemical evaluation which included: bimanual examination, PAP smear and transvaginal sonography, breast examination and mammography, thyroid-liver-renal function as well as blood coagulation tests and bone densitometry. Criteria for inclusion in the study were an endometrial thickness ≤5mm and the absence of a history of gynecological malignancy, ischemic heart disease, thromboembolism, diabetes mellitus and non-treated thyroid dysfunction. Ovariectomized women or women taking lipid-lowering or antihypertensive drugs were not included in the study. All subjects signed an informed consent and approval of the Institutional Review Board and of the Ethics Committee of Aretaieion Hospital was obtained.

**Protocol**

Participating women were evaluated in a cross-sectional design. In every subject a detailed medical history was recorded including regimen and duration of replacement therapy. Blood pressure, weight and height were measured in the morning in light clothing and Body Mass Index (BMI) was computed. Subsequently, fasting blood samples were drawn between 9:00 and 10:00 a.m. for the determination of FSH, estradiol, total testosterone, SHBG, Δ4A, DHEAS and homocysteine levels. Samples were immediately centrifuged and serum was stored at −30°C until assayed.
**Hormone assays**

FSH was measured with the Microparticle Enzyme Immunoassay kits: FSH, Abbott Axsym and LH Abbott Axsym, respectively, on Axsym analyzer (Abbott Laboratories, USA). The total coefficient of variation ranged from 5.3% to 8.5%. Estriadiol was measured with the commercial Enzyme Immunoassay kit: DSK-10-4300, Diagnostic Systems Laboratories Inc. The total CV Ranged from 4.3-6.1% and the sensitivity was 8 pg/ml. Δ4-Androstendione was measured with the ELISA kit: IBL “Androstendione ELISA” (IBL GmbH, Hamburg, Germany). The interassay %CV ranged from 6.5 to 8.1. Total Testosterone, DHEAS and SHBG were measured with the DPC kits: “Immulite Total Testosterone”, “Immulite DHEA-SO4” and “Immulite SHBG” on Immulite analyzer (Diagnostic Products Corporation, Los Angeles, USA). The total %CV ranged from 8.0% to 16.0%, 8.1% to 15% and 4.1% to 9.2%, respectively, as measured in our Laboratory. Free Androgen Index (FAI) was calculated using total testosterone and SHBG values by the following equation: FAI=testosterone (ng/ml) x 3.47 x 100/SHBG (nmol/l). Total Hcy concentrations were measured by the Abbott commercial kit: IMx Homocysteine (Abbott Lab, Abbott Park, IL 60064). The total CV (%) and the sensitivity were: 4.3% and 0.5 μmol/l, respectively.

**Statistics**

Statistical analysis was performed by SPSS Version 11.5 (Statistical Package for the Social Sciences, Chicago, Illinois). Associations between demographic characteristics, serum sex hormones and homocysteine values were assessed by Pearson correlation analysis. Means of continuous variables were compared between groups by the Students-t test for unpaired data. Nominal variables were compared between groups by the chi-square test. Adjustments for possible confounding factors were performed by Analysis of Covariance (ANCOVA). Statistical significance was set at the 0.05 level.

**RESULTS**

Demographic characteristics as well as mean levels of serum Hcy and sex hormones of the 484 women participating in the study are presented in Table 1. Estradiol, testosterone and FAI were not normally distributed and thus were logarithmically transformed for further processing.

Mean levels of serum Hcy according to lifestyle factors and family history of CVD are presented in Table 2. Current smokers versus non-smokers and women with a family history of CVD versus those without a family history of CVD tended to have higher serum Hcy levels, but these differences are suggestive (p=0.08 and p=0.09, respectively). Alcohol intake and physical exercise did not associate with serum Hcy (p >0.20).

Correlation coefficients between serum Hcy and age, years since menopause (YSM), BMI and sex hormones are presented in Table 3. Serum FSH correlated positively and serum estradiol negatively with circulating Hcy (r=0.23, p=0.01 and r=-0.27, p=0.02, respectively). This association persisted after adjustment for age, YSM and BMI. Serum FSH increased, while serum estradiol decreased linearly with increasing Hcy quartiles (Figure 1). Circulating Hcy did not associate with any of the androgens assessed (Table 3).

**DISCUSSION**

In this study estrogen levels among non-treated postmenopausal women correlated inversely, while serum FSH correlated positively with Hcy levels. No association between Hcy levels and the androgen levels was disclosed.

Sex steroids may be important in modulating Hcy metabolism. Estradiol appears to relate to the estrogen status of woman and to the phases of the menstrual cycle. It has been reported that Hcy is lower in premenopausal as compared to postmenopausal women and to increase significantly following menopause. Other investigators, however have not reported a menopause-related influence on Hcy levels. Furthermore, it is still unclear whether the higher postmenopausal Hcy levels detected in certain studies are related to an effect of estrogen deficiency on Hcy metabolism or to an age-related disruption in the remethylation and transsulfuration metabolic pathways.
Table 1. Baseline demographic characteristics and hormone levels of the 484 healthy postmenopausal women included in the study.

<table>
<thead>
<tr>
<th>Numeric Variables</th>
<th>Mean ± SD</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55.3 ± 5.6</td>
<td>43</td>
<td>69</td>
</tr>
<tr>
<td>Years since menopause (YSM)</td>
<td>5.6 ± 5.4</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>BMI (Kg/m2)</td>
<td>25.4 ± 3.6</td>
<td>17.6</td>
<td>40.6</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>69.4 ± 28.8</td>
<td>21</td>
<td>168</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>14.9** ± 8.2</td>
<td>&lt;8*</td>
<td>36</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>0.60** ± 0.51</td>
<td>&lt;0.2*</td>
<td>2.8</td>
</tr>
<tr>
<td>Free androgen index (FAI)</td>
<td>2.9** ± 3.1</td>
<td>0.2</td>
<td>12.3</td>
</tr>
<tr>
<td>Δ4A (ng/dl)</td>
<td>104 ± 48</td>
<td>11</td>
<td>355</td>
</tr>
<tr>
<td>DHEAS (ng/ml)</td>
<td>912 ± 439</td>
<td>105</td>
<td>2510</td>
</tr>
<tr>
<td>Homocysteine (μmol/l)</td>
<td>10.8 ± 3.3</td>
<td>5.5</td>
<td>26.0</td>
</tr>
</tbody>
</table>

Nominal variables N (%)

- Current smoking (>10 cigarettes/day) 117 (24.2)
- Physical exercise (>3h/week) 90 (18.6)
- Daily alcohol intake (>12g/day) 13 (2.7)
- Family history of CVD (1st degree relative) 107 (22.1)

(To convert: testosterone from ng/dl to nmol/L, estradiol from pg/ml to pmol/L, δ4Androstendion from ng/ml to nmol/L and DHEAS from μg/dl to μmol/L please multiply by conversion factor 0.0347, 3.67, 0.0349 and 0.00272 respectively)

* undetectable values

**median values are presented due to skewed distribution

Table 2. Mean (SD) serum homocysteine levels (μmol/l) according to lifestyle factors and family history of cardiovascular disease in the 484 healthy postmenopausal women: Comparisons between the groups (Yes or No).

<table>
<thead>
<tr>
<th>Homocysteine levels</th>
<th>Mean (SD)</th>
<th>Mean (SD)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smoking (&gt;10 cigarettes/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=117) vs No (n=367)</td>
<td>11.0 (3.5)</td>
<td>vs</td>
<td>0.08</td>
</tr>
<tr>
<td>Physical exercise (&gt;3h/week)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=90) vs No (n=394)</td>
<td>11.0 (3.7)</td>
<td>vs</td>
<td>0.23</td>
</tr>
<tr>
<td>Daily alcohol intake (&gt;12g/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=13) vs No (n=471)</td>
<td>10.9 (2.9)</td>
<td>vs</td>
<td>0.56</td>
</tr>
<tr>
<td>Family history of CVD (1st degree relative)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=107) vs No (n=377)</td>
<td>10.8 (2.7)</td>
<td>vs</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*p: students t-test for unpaired observations.

Menopausal women appears to be age-related; Hcy levels were similar to those reported for premenopausal women, remained unchanged until the age of 60 years, fluctuating around 10μmol/l, and increased significantly in women older than 65 years.

In the present study endogenous estrogen status seems to influence circulating Hcy in postmenopausal women, as indicated by both FSH and estradiol associations. The inverse correlation between Hcy and estrogen levels, as well as the positive correlation between Hcy and FSH, most likely indicate that HT administration would decrease circulating Hcy in postmenopausal women. With the exception of one RCT reported by Van Baal et al., evidence of
Endogenous sex steroids and circulating homocysteine in healthy Greek postmenopausal women

39Endogenous sex steroids and circulating homocysteine in healthy Greek postmenopausal women

ulture regarding the effect of transdermal HT administration. An observational study, and a recent RCT have reported no effect of estrogens on Hcy, while two observational studies associated transdermal HT with a significant Hcy decrease. We cannot, therefore, draw any firm conclusion either on the effect of estrogens on Hcy or the possible role of hepatic metabolism in modulating Hcy levels.

No study has yet elucidated the role of progestin in the combined HT. Progestins, depending on their chemical structure, may enhance, attenuate or even reverse estrogen's effect. Androgenic progestins are known to modify the lipids profile. Mijatovic et al and Van Baal investigating dydrogesterone and trimethisterone, respectively, reported that these progestins potentiate estrogen's lowering effect on Hcy, while Evio attributed the absence of an effect to the presence of NETA. However, of five studies investigating the effect of E2 + NETA on Hcy, only one did not demonstrate a decreasing effect. A more precise methodology, as suggested by Lobo, could possibly clarify the role of the progestin.

In the present study DHEAS, testosterone as well as FAI were not related with serum Hcy. If indeed increased estrogen levels associate with decreased Hcy, then it is logical to assume that HT given to symptomatic women in menopause of recent onset might modulate favorably cardiovascular risk. However, it remains to be proven whether lowering Hcy levels is associated with a lower cardiovascular risk. To date, no study has investigated whether the decrease in Hcy levels by HT translates clinically to cardiovascular risk reduction.

Figure 1. Mean serum FSH (IU/L) and estradiol (pg/ml) in 484 postmenopausal women not on hormone therapy according to serum homocysteine quartiles. p values from ANOVA test for linear trend.

Table 3. Pearson simple and partial correlation coefficients between serum homocysteine and sex steroids.

<table>
<thead>
<tr>
<th>Variable</th>
<th>r</th>
<th>p</th>
<th>partial r</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.18</td>
<td>0.048</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Years since menopause (YSM)</td>
<td>0.10</td>
<td>0.17</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>0.09</td>
<td>0.56</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>0.23</td>
<td>0.01</td>
<td>0.19</td>
<td>0.03</td>
</tr>
<tr>
<td>Estradiol (pg/ml)**</td>
<td>-0.27</td>
<td>0.02</td>
<td>-0.20</td>
<td>0.04</td>
</tr>
<tr>
<td>Testosterone (ng/ml)**</td>
<td>0.07</td>
<td>0.44</td>
<td>0.06</td>
<td>0.39</td>
</tr>
<tr>
<td>FAI**</td>
<td>0.12</td>
<td>0.17</td>
<td>0.09</td>
<td>0.26</td>
</tr>
<tr>
<td>Δ4A (ng/dl)</td>
<td>-0.11</td>
<td>0.13</td>
<td>-0.04</td>
<td>0.25</td>
</tr>
<tr>
<td>DHEAS (ng/dl)</td>
<td>-0.09</td>
<td>0.12</td>
<td>-0.07</td>
<td>0.36</td>
</tr>
</tbody>
</table>

* p values after adjustment for age, YSM and BMI
(To convert: testosterone from ng/dl to nmol/L, estradiol from pg/ml to pmol/L, Δ4Androstendion from ng/ml to nmol/L and DHEAS from µg/dl to µmol/L, please multiply by conversion factor 0.0347, 3.67, 0.0349 and 0.00272, respectively)

** logarithmically transformed due to skewed distribution
REFERENCES


and women in the NHANES III. J Nutr 130: 3073-3076.