The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/20871

Please be advised that this information was generated on 2019-03-02 and may be subject to change.
Plasma homocysteine and menopausal status*

G. H. J. BOERS,‡ L. A. SCHELLEKENS,* C. M. G. THOMAS* & T. K. A. B. ESKES* Departments of
*Obstetrics and Gynaecology, †Paediatrics and ‡Medicine, University Hospital Nijmegen St Radboud,
Nijmegen, the Netherlands

Received 5 September 1994 and in revised form 13 March 1995; accepted 21 March 1995

Abstract. The aim of the study was to measure the concentrations of plasma homocysteine in premeno-
pausal and postmenopausal women, and to examine a possible relationship between plasma homocysteine
and oestrogen status. Homocysteine metabolism was studied by a standardized oral methionine loading
test, and oestrogen status was assessed by the measurement of serum 17β-oestradiol. Forty-six premenopausal
and 26 postmenopausal healthy women without a history of vascular disease or adverse preg-
nancy outcome were recruited by public advertisement. The main outcome measures were the concentra-
tions of fasting and postmethionine plasma homocysteine, and serum 17β-oestradiol. Fasting plasma
homocysteine concentrations (mean ± SD) were significantly higher in postmenopausal women as com-
pared to premenopausal women (12 ± 4 µmol L⁻¹ and 10 ± 3 µmol L⁻¹, respectively) as well as post-
methionine plasma homocysteine concentrations (46 ± 16 µmol L⁻¹ and 32 ± 9 µmol L⁻¹, respec-
tively). In premenopausal women, postmethionine plasma homocysteine was negatively and significantly
correlated to serum 17β-oestradiol (r = —0·34). It is concluded that plasma homocysteine concentrations,
both fasting and after methionine loading, are significantly higher in postmenopausal women than in pre-
menopausal women. In premenopausal women, the higher concentrations of serum 17β-oestradiol may
account in part for the lower concentrations of postmethionine plasma homocysteine.

Keywords. Homocysteine, menopause, oestradiol, vitamins.

Introduction

Homocysteine is a thiol-containing amino acid that
results from the demethylation of methionine. Homo-
cysteine is metabolized by transsulfuration via
cystathionine to cysteine or by remethylation to
methionine. The conversion to cystathionine is cata-
ysed by the enzyme cystathionine β-synthase in a
reaction requiring pyridoxal-5'-phosphate (PLP, an
active form of vitamin B6) as a cofactor. In the
remethylation of homocysteine to methionine, the
methyl group is donated by either 5-methyltetrahy-
drofolate, requiring methylcobalamin as a cofactor,
or betaine [1].

Homocysteine in blood is mainly bound in disul-
fides to proteins and to cysteine. A minor fraction
constitutes of reduced homocysteine and the disulfide
homocysteine–homocysteine (homocystine). Total
homocysteine is defined as the sum of all homocys-
teine species liberated by reduction [2].

Hyperhomocysteinaemia, i.e. the mild elevation of
homocysteine in blood, is a well-established risk
factor of premature vascular disease [3,4]. Recently,
we have reported significantly elevated concentrations
of plasma homocysteine in women with unexplained
recurrent early pregnancy loss or a neural tube defect-
fected pregnancy [5,6]. Hyperhomocysteinaemia
may also be a risk factor in women who have suffered
from placental abruption [7].

There are several reports indicating that blood
homocysteine concentrations determined as homocys-
teine–cysteine mixed disulfide and homocystine [8–
10], protein-bound homocyst(e)ine [11], or total
homocysteine [12–16] are lower in women as com-
pared to men. Furthermore, in premenopausal
women the serum concentrations of homocysteine–
cysteine mixed disulfide and homocysteine were
demonstrated to be significantly lower than in post-
menopausal women [9,10]. It was proposed that a
uniquely efficient methionine metabolism may
account for the lower incidence of vascular disease
in women before menopause [9]. In contrast, it was
recently reported that the plasma concentrations of
free and total homocysteine are almost similar in
women and men, and independent of menopausal
status [17]. The aim of the present study was to

* Presented at the 7th International Congress on the Menopause,
Stockholm, Sweden, 20–24 June 1993 and at the International
Symposium on Women's Health in Menopause, Milan, Italy, 26–
29 September 1993.

Correspondence: M. G. A. J. Wouters MD, Department of
Obstetrics and Gynaecology, University Hospital Nijmegen St
Radboud, PO Box 9101, 6500 HB Nijmegen, the Netherlands.

© 1995 Blackwell Science Ltd
measure the concentrations of plasma total homocysteine in premenopausal and postmenopausal women without a history of vascular disease or adverse pregnancy outcome, and to examine a possible relationship between plasma homocysteine and oestrogen status.

**Subjects and methods**

**Subjects**

Homocysteine metabolism was studied in 46 premenopausal women (aged 27–44 years) and in 26 postmenopausal women (aged 50–60 years), who were recruited by public advertisement. Premenopausal women were routinely studied approximately 1 week before the expected onset of the next menstrual period. They were not pregnant at the time of investigation. Postmenopausal women had reported the absence of menses for at least 1 year. Their postmenopausal status was confirmed by the measurement of serum follicle-stimulating hormone. All women (n = 72) had delivered at least one liveborn, healthy child, and had no history of (recurrent) spontaneous abortion, fetal death, neural tube defect–affected pregnancy, or placental abruption. They were healthy and had not experienced renal, liver or vascular disease. Serum creatinine, serum alanine aminotransferase, and serum aspartate aminotransferase were within the normal range. Participants were not allowed to take oral contraceptives, hormonal or vitamin supplements, or any other medication that could possibly interfere with homocysteine metabolism for at least 3 months prior to the study. The study was approved by the ethical committee of the University Hospital Nijmegen St Radboud, Nijmegen, the Netherlands. Written informed consent was obtained from all women before participation.

**Investigation procedure**

Homocysteine metabolism was studied by a standardized oral methionine loading test as described elsewhere [5]. In summary, after an overnight fast venous blood samples were drawn to measure the concentrations of plasma homocysteine, serum and red cell folate, serum vitamin B12, and whole blood PLP. Then, L-methionine, 0·1 g (0·7 mmol) per kg body weight, was administered orally in 200 mL of orange juice. The plasma homocysteine concentration was measured again 6 h after methionine loading. The oestrogen status of all participants was assessed on the same day by the fasting serum concentration of 17β-oestradiol.

**Sample preparation and analysis**

Blood samples for the quantitation of plasma homocysteine were drawn in ethylenediamine tetraacetate vacuum tubes of 4 mL, and centrifuged within 30 min at 3000 g for 10 min. The plasma was separated and stored at −20°C until analysis. Total homocysteine concentrations were measured by the high-performance liquid chromatography (HPLC) technique with fluorimetric detection, essentially according to Fiskerstrand et al. [18]. The lower limit of detection was 0·5 μmol L−1; the intra-assay and interassay coefficients of variation were both below 5%. Serum concentrations of 17β-oestradiol were measured with a highly specific inhouse radioimmunoassay procedure [19]. The lower limit of detection was 38 pmol L−1. Dry and heparinized vacutainer tubes of 10 mL were used for collecting venous samples to assay the concentrations of folate in serum and red cells, vitamin B12 in serum, and PLP in whole blood. Folate and vitamin B12 concentrations were measured simultaneously with Dualcount SPB (Solid Phase Boil) Radioassay (Diagnostic Products Corporation, Los Angeles, CA, USA) [20]. Determination of PLP was performed by HPLC technique [21].

**Statistical analysis**

Results are given as mean ± SD. In assessing statistical significance, the Wilcoxon rank sum test and Spearman’s rank correlation were used. P values were two-tailed and P < 0·05 was considered significant.

**Results**

The mean concentrations of plasma homocysteine, both fasting and after methionine loading, were significantly higher in postmenopausal women as compared to premenopausal women (Table 1). Figure 1 depicts the individual fasting and postmethionine plasma homocysteine concentrations of both groups studied.

Table 1. Concentrations of plasma homocysteine, fasting and after methionine loading, serum 17β-oestradiol and blood vitamins in premenopausal and postmenopausal women

<table>
<thead>
<tr>
<th></th>
<th>Fasting plasma homocysteine (μmol L−1)</th>
<th>Postmethionine plasma homocysteine (μmol L−1)</th>
<th>Serum 17β-oestradiol (pmol L−1)</th>
<th>Serum folate (nmol L−1)</th>
<th>Red cell folate (nmol L−1)</th>
<th>Serum vitamin B12 (pmol L−1)</th>
<th>Whole blood PLP (nmol L−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Premenopausal</strong></td>
<td>10 ± 3</td>
<td>32 ± 9</td>
<td>761 ± 284</td>
<td>14 ± 4</td>
<td>513 ± 144</td>
<td>285 ± 109</td>
<td>53 ± 20</td>
</tr>
<tr>
<td><strong>women n = 46</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Postmenopausal</strong></td>
<td>12 ± 4†</td>
<td>46 ± 16†</td>
<td>76 ± 27†</td>
<td>16 ± 5†</td>
<td>569 ± 159</td>
<td>226 ± 655</td>
<td>46 ± 10</td>
</tr>
<tr>
<td><strong>women n = 26†</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values are expressed as mean ± SD; † n = 24 in case of serum 17β-oestradiol; ‡ significantly different as compared to premenopausal women, P < 0·05.
HOMOCYSTEINE AND MENOPAUSAL STATUS

Figure 1. Individual values of plasma homocysteine, fasting (×) and after methionine loading (△), in premenopausal (n = 46) and postmenopausal women (n = 26).

Discussion

Plasma homocysteine, serum 17β-oestradiol, and blood vitamin concentrations were studied in 46 healthy premenopausal and 26 healthy postmenopausal women. Our results clearly demonstrate that plasma homocysteine concentrations, both fasting and after methionine loading, are higher in postmenopausal women as compared to premenopausal women. The results of our study are in agreement with those of other reports in which homocysteine was measured either as free disulfides, before as well as after methionine loading [9,10], or as protein-bound homocyst(e)ine in the fasting state [11]. In contrast, Andersson et al. did not find significant differences in plasma total homocysteine concentrations, either fasting or after methionine loading, relating to menopausal status [17]. Currently, we have no clear-cut explanation for this discrepancy, although some differences in subject selection may be of importance.

Several studies have demonstrated a significantly positive age-correlation of plasma homocysteine in women [11,13,14,16,22]. Our results suggest an age-dependent increase of plasma homocysteine as well. Neither in premenopausal nor in postmenopausal women, however, was plasma homocysteine significantly correlated to age, possibly due to the narrow ranges of age (27–44 and 50–60 years, respectively). Interestingly, Kang et al. have reported an abrupt increase of plasma homocysteine among women after 50 years of age, suggesting a negative relationship with hormonal changes after menopause [11]. Our study is probably the first to report a significantly negative correlation between the concentrations of postmethionine plasma homocysteine and serum 17β-oestradiol (Table 2, Fig. 2). Remarkably, this relationship was found in premenopausal, but not in postmenopausal, women. We speculate that in premenopausal women, the higher concentrations of serum 17β-oestradiol account in part for the lower concentrations of postmethionine plasma homocysteine, whereas in postmenopausal women, the range of serum 17β-oestradiol is too small to detect a similar relationship. This view is in line with the observed reduction of blood homocysteine in women during no significant correlations between plasma homocysteine and oestrogen status were found. Neither in premenopausal nor in postmenopausal women, fasting and postmethionine plasma homocysteine were significantly correlated to age or body weight. In premenopausal women, both fasting and postmethionine plasma homocysteine were negatively and significantly correlated to serum folate, but not to the other vitamins. In postmenopausal women, fasting plasma homocysteine correlated negatively and significantly to the vitamins studied, except for PLP, whereas postmethionine plasma homocysteine was negatively and significantly correlated to serum folate, but not to the other vitamins.
pregnancy, a condition which is characterized by a high concentration of circulating oestrogens [23,24]. Additionally, we have recently reported that high fasting serum homocysteine concentrations may decrease by natural oestrogen supplementation after menopause [25]. Synthetic oestrogens, like ethinyl-oestradiol in oral contraceptives, may affect homocysteine metabolism as well, although data are conflicting [21,26].

Serum folate and serum vitamin B12 are important determinants of fasting plasma homocysteine in healthy subjects [15-17], in patients with vascular disease [27,28], and in women with recurrent spontaneous abortion [5]. This is confirmed by the present results, except that in premenopausal women the concentrations of fasting plasma homocysteine were not correlated to those of serum vitamin B12 (Table 2). In postmenopausal women, unlike premenopausal women, fasting plasma homocysteine concentrations were negatively and significantly correlated to red cell folate concentrations (Table 2). Altogether, these data suggest that, in postmenopausal women, fasting plasma homocysteine is more dependent on blood vitamins as compared to premenopausal women. In a recent study of an elderly population aged 67-96 years, it appeared that a marginal or manifest vitamin deficiency may contribute to two-thirds of the cases of high plasma homocysteine [29].

Postmethionine plasma homocysteine was negatively and significantly correlated to serum folate, both in premenopausal and postmenopausal women. However, the increase of plasma homocysteine after methionine loading and serum folate were reported to be not significantly correlated [17,27].

The risk of cardiovascular disease in women

Table 2. Correlations between plasma homocysteine, fasting and after methionine loading, and serum 17β-oestradiol, age, body weight, and blood vitamins in premenopausal and postmenopausal women*

<table>
<thead>
<tr>
<th></th>
<th>Fasting plasma homocysteine</th>
<th>Postmethionine plasma homocysteine</th>
<th>Fasting plasma homocysteine</th>
<th>Postmethionine plasma homocysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 17β-oestradiol</td>
<td>-0.06</td>
<td>-0.34†</td>
<td>+0.24</td>
<td>+0.28</td>
</tr>
<tr>
<td>Age</td>
<td>+0.11</td>
<td>-0.05</td>
<td>+0.07</td>
<td>+0.10</td>
</tr>
<tr>
<td>Body weight</td>
<td>-0.15</td>
<td>+0.07</td>
<td>+0.01</td>
<td>+0.08</td>
</tr>
<tr>
<td>Serum folate</td>
<td>-0.43‡</td>
<td>-0.48‡</td>
<td>-0.78‡</td>
<td>-0.39‡</td>
</tr>
<tr>
<td>Red cell folate</td>
<td>+0.07</td>
<td>+0.11</td>
<td>-0.49‡</td>
<td>-0.37</td>
</tr>
<tr>
<td>Serum vitamin B12</td>
<td>+0.08</td>
<td>+0.07</td>
<td>-0.50‡</td>
<td>-0.08</td>
</tr>
<tr>
<td>Whole blood PLP</td>
<td>+0.03</td>
<td>+0.04</td>
<td>-0.08</td>
<td>+0.03</td>
</tr>
</tbody>
</table>

*Values are Spearman’s rank correlation coefficients; † n = 24 in case of serum 17β-oestradiol; ‡ significant correlation, P < 0.05.
increases rapidly after menopause. Higher concentrations of plasma homocysteine are associated with a higher risk of cardiovascular disease [4]. Our data support the hypothesis that, in women after menopause, the higher concentrations of plasma homocysteine contribute to the higher risk of cardiovascular disease.

It is concluded that plasma homocysteine concentrations, both fasting and after methionine loading, are significantly higher in postmenopausal women than in premenopausal women. In premenopausal women, the higher concentrations of serum 17β-oestradiol may account in part for the lower concentrations of postmenopausal plasma homocysteine.

Acknowledgments

The authors wish to thank all the women who partici­pated in the study. We also acknowledge Maria T. W. te Poele-Pothoff for her assistance in laboratory analysis, Martijn F. G. Segers BSc, for his technical support, and Nelleke J. Hamel-van Bruggen, research nurse, for her assistance in data collection. Supported by grant number 28.1006.1 from Pravencentiefonds, The Hague, the Netherlands.

References