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## Plasma homocysteine and menopausal status\*

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**Abstract.** The aim of the study was to measure the concentrations of plasma homocysteine in premenopausal 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  $17\beta$ -oestradiol. Forty-six premenopausal and 26 postmenopausal healthy women without a history of vascular disease or adverse pregnancy outcome were recruited by public advertisement. The main outcome measures were the concentrations of fasting and postmethionine plasma homocysteine, and serum  $17\beta$ -oestradiol. Fasting plasma homocysteine concentrations (mean  $\pm$  SD) were significantly higher in postmenopausal women as compared to premenopausal women ( $12 \pm 4 \mu\text{mol L}^{-1}$  and  $10 \pm 3 \mu\text{mol L}^{-1}$ , respectively) as well as postmethionine plasma homocysteine concentrations ( $46 \pm 16 \mu\text{mol L}^{-1}$  and  $32 \pm 9 \mu\text{mol L}^{-1}$ , respectively). In premenopausal women, postmethionine plasma homocysteine was negatively and significantly correlated to serum  $17\beta$ -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 premenopausal women. In premenopausal women, the higher concentrations of serum  $17\beta$ -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

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results from the demethylation of methionine. Homocysteine is metabolized by transsulfuration via cystathionine to cysteine or by remethylation to methionine. The conversion to cystathionine is catalysed by the enzyme cystathionine  $\beta$ -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-methyltetrahydrofolate, requiring methylcobalamin as a cofactor, or betaine [1].

Homocysteine in blood is mainly bound in disulfides 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 homocysteine 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-affected 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 homocysteine–cysteine mixed disulfide and homocystine [8–10], protein-bound homocyst(e)ine [11], or total homocysteine [12–16] are lower in women as compared to men. Furthermore, in premenopausal women the serum concentrations of homocysteine–cysteine mixed disulfide and homocystine were demonstrated to be significantly lower than in postmenopausal 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

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  $17\beta$ -oestradiol.

### Sample preparation and analysis

Blood samples for the quantitation of plasma homocysteine were drawn in ethylenediamine tetraacetate vacutainer tubes of 4 mL, and centrifuged within

30 min at 3000 *g* for 10 min. The plasma was separated and stored at  $-20^{\circ}\text{C}$  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  $0.5 \mu\text{mol L}^{-1}$ ; the intra-assay and interassay coefficients of variation were both below 5%. Serum concentrations of  $17\beta$ -oestradiol were measured with a highly specific inhouse radioimmunoassay procedure [19]. The lower limit of detection was  $38 \text{ pmol L}^{-1}$ . 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  $\pm$  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.

In premenopausal women, serum  $17\beta$ -oestradiol concentrations ranged from 330 to  $1500 \text{ pmol L}^{-1}$ ,

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

	Premenopausal women $n = 46$	Postmenopausal women $n = 26^{\dagger}$
Fasting plasma homocysteine ( $\mu\text{mol L}^{-1}$ )	$10 \pm 3$	$12 \pm 4^{\ddagger}$
Postmethionine plasma homocysteine ( $\mu\text{mol L}^{-1}$ )	$32 \pm 9$	$46 \pm 16^{\ddagger}$
Serum $17\beta$ -oestradiol ( $\text{pmol L}^{-1}$ )	$761 \pm 284$	$76 \pm 27^{\ddagger}$
Serum folate ( $\text{nmol L}^{-1}$ )	$14 \pm 4$	$16 \pm 5^{\ddagger}$
Red cell folate ( $\text{nmol L}^{-1}$ )	$513 \pm 144$	$569 \pm 159$
Serum vitamin B12 ( $\text{pmol L}^{-1}$ )	$285 \pm 109$	$226 \pm 85^{\ddagger}$
Whole blood PLP ( $\text{nmol L}^{-1}$ )	$53 \pm 20$	$46 \pm 10$

\* Values are expressed as mean  $\pm$  SD;  $^{\dagger} n = 24$  in case of serum  $17\beta$ -oestradiol;  $^{\ddagger}$  significantly different as compared to premenopausal women,  $P < 0.05$ .

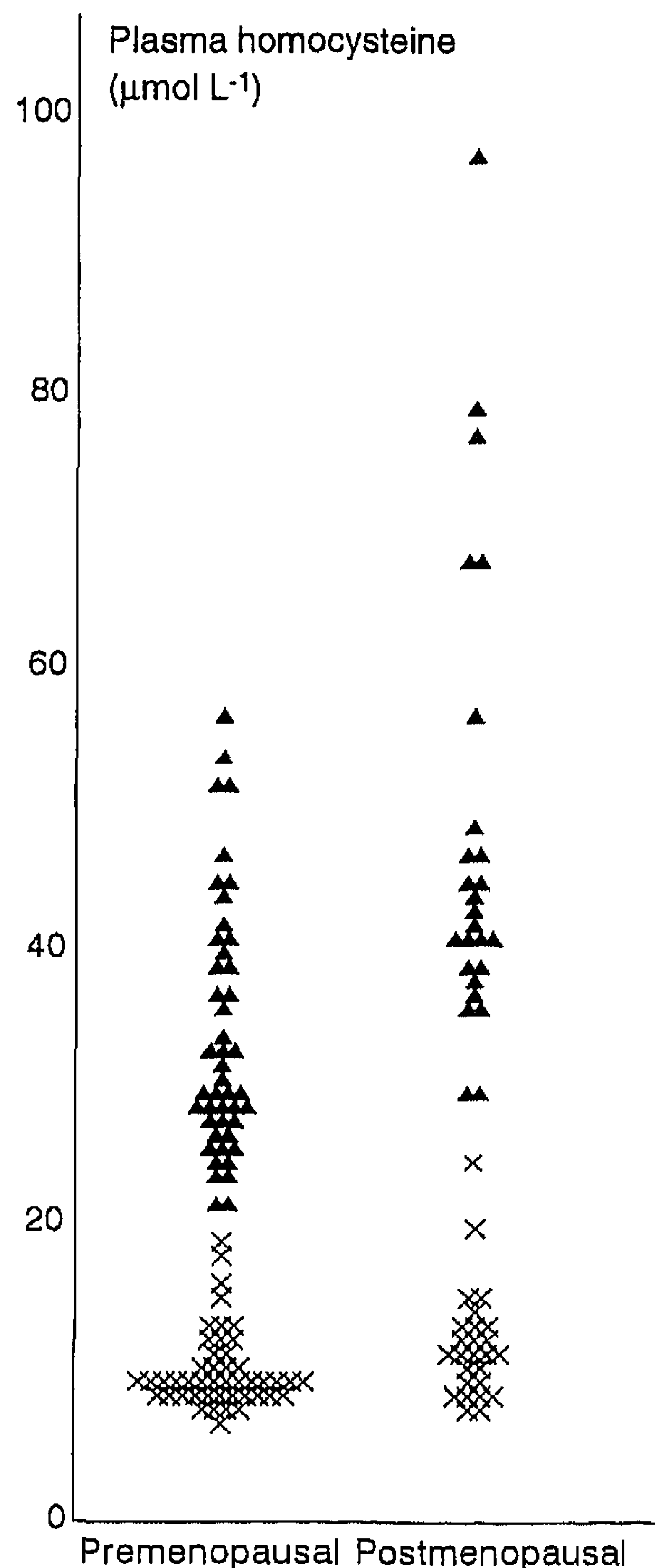


Figure 1. Individual values of plasma homocysteine, fasting ( $\times$ ) and after methionine loading ( $\blacktriangle$ ), in premenopausal ( $n = 46$ ) and postmenopausal women ( $n = 26$ ).

whereas in postmenopausal women these concentrations did not exceed  $160 \text{ pmol L}^{-1}$ . In two women of the postmenopausal group, serum  $17\beta$ -oestradiol concentrations were below the detection limit (Table 1).

The mean serum and red cell concentrations of folate were higher in postmenopausal women than in premenopausal women, but the difference was only significant in the case of serum folate (Table 1). Mean serum vitamin B12 was significantly lower in postmenopausal women than in premenopausal women (Table 1). The mean concentrations of whole blood PLP were not significantly different between groups (Table 1).

Table 2 lists the Spearman's rank correlation coefficients between plasma homocysteine, fasting as well as after methionine loading, and serum  $17\beta$ -oestradiol, age, body weight, and blood vitamins in premenopausal and postmenopausal women. In premenopausal women, postmethionine plasma homocysteine, unlike fasting plasma homocysteine, was negatively and significantly correlated to serum  $17\beta$ -oestradiol (Fig. 2). In postmenopausal women,

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.

## Discussion

Plasma homocysteine, serum  $17\beta$ -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  $17\beta$ -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  $17\beta$ -oestradiol account in part for the lower concentrations of postmethionine plasma homocysteine, whereas in postmenopausal women, the range of serum  $17\beta$ -oestradiol is too small to detect a similar relationship. This view is in line with the observed reduction of blood homocysteine in women during

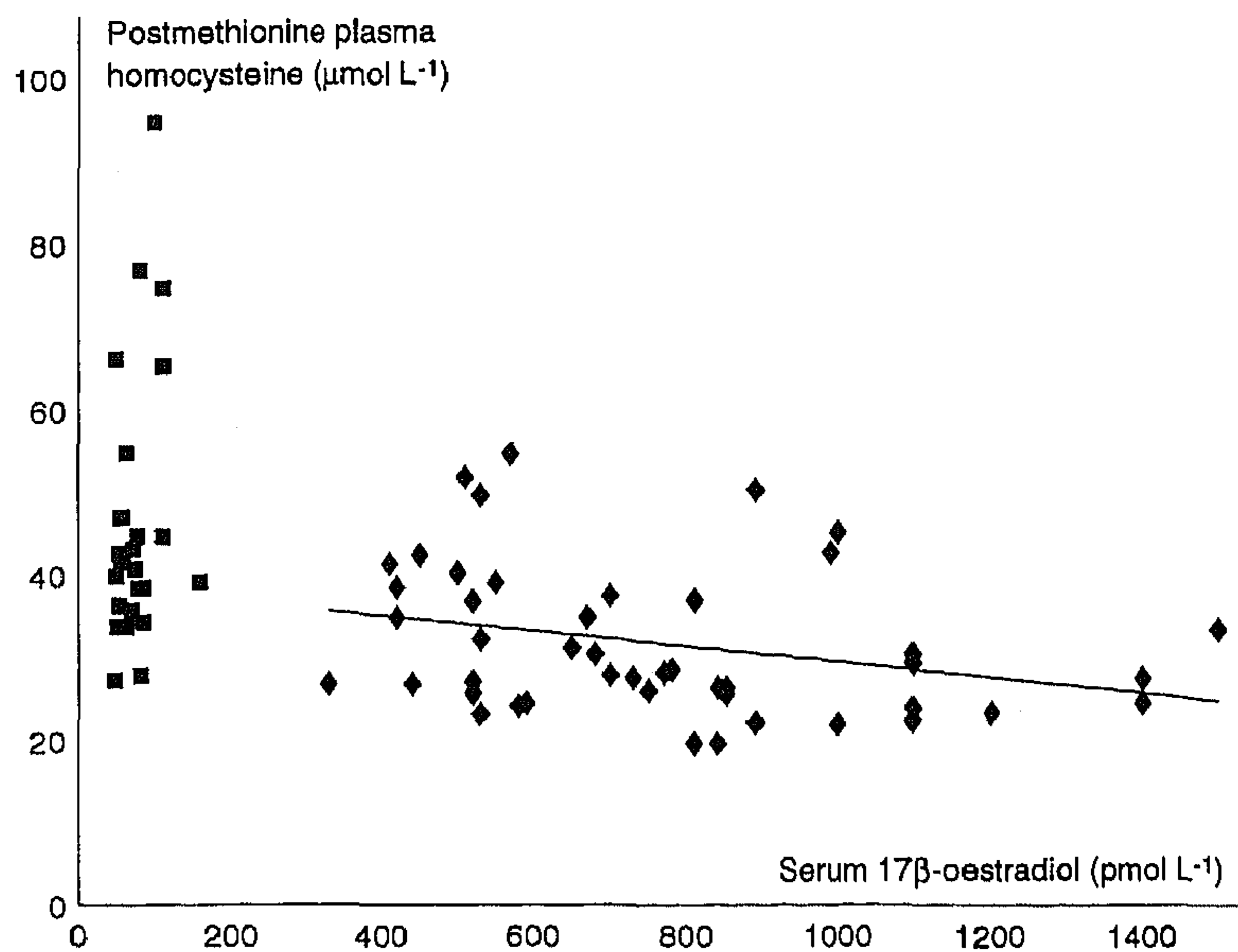


Figure 2. Scatter diagram of postmethionine plasma homocysteine and serum  $17\beta$ -oestradiol in premenopausal ( $\blacklozenge$ ;  $r = -0.34$ ,  $P < 0.05$ ) and postmenopausal women ( $\blacksquare$ ;  $r = 0.28$ ,  $P = 0.18$ ).

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. This observation has not been described by others. 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  $17\beta$ -oestradiol, age, body weight, and blood vitamins in premenopausal and postmenopausal women\*

	Premenopausal women $n = 46$		Postmenopausal women $n = 26^\dagger$	
	Fasting plasma homocysteine	Postmethionine plasma homocysteine	Fasting plasma homocysteine	Postmethionine plasma homocysteine
Serum $17\beta$ -oestradiol	-0.06	-0.34‡	+0.24	+0.28
Age	+0.11	-0.05	+0.07	+0.10
Body weight	-0.15	+0.07	+0.01	+0.08
Serum folate	-0.43‡	-0.48‡	-0.78‡	-0.39‡
Red cell folate	+0.07	+0.11	-0.49‡	-0.37
Serum vitamin B12	+0.08	+0.07	-0.50‡	-0.08
Whole blood PLP	+0.03	+0.04	-0.08	+0.03

\* Values are Spearman's rank correlation coefficients;  $^\dagger n = 24$  in case of serum  $17\beta$ -oestradiol;  $^\ddagger$  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  $17\beta$ -oestradiol may account in part for the lower concentrations of postmethionine plasma homocysteine.

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