Plasma total homocysteine levels in postmenopausal women with unstable coronary artery disease

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Abstract

An elevated plasma total homocysteine (tHcy) level is considered a risk factor for coronary artery disease (CAD), but the relationship between plasma tHcy and well-defined CAD in women is still unclear. Plasma tHcy concentrations and the covariates serum folate, vitamin B12, and creatinine were analysed in 157 angiographically examined postmenopausal women with unstable CAD and in 101 healthy controls. At coronary angiography, 16% had normal vessels and 84% had coronary atherosclerosis. Mean plasma tHcy concentration (μmol/l, 95% confidence interval) did not differ in patients compared to controls (13.1 (12.3–13.8) vs. 12.5 (11.6–13.5)) or in patients with or without coronary atherosclerosis (13.3 (12.4–14.1) vs. 12.0 (10.8–13.2)). A trend to an increasing plasma tHcy with increasing degree of coronary atherosclerosis was attenuated after adjustment for age and the previous mentioned covariates. Odds ratio for the risk of coronary artery disease and coronary atherosclerosis in hyperhomocysteinemic patients (≥90th percentile in controls) was approximately 3. However, the confidence interval included unity in half of the groups and the significance was therefore difficult to judge. Receiver operating characteristics showed age to be the only variable with a significant discriminatory ability regarding the presence of coronary atherosclerosis (area 0.77). Mild hyperhomocysteinemia seems not to be related to the risk of unstable CAD in postmenopausal women. The trend towards higher plasma tHcy with increasing degree of coronary atherosclerosis may be a marker of the disease. In future studies adjustment for age and the other three covariates should be considered. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Homocysteine; Women; Unstable coronary artery disease; Coronary angiography; Odds ratio; Covariates; ROC curve

1. Introduction

In homocysteinurias, severely increased plasma total homocysteine concentration (tHcy) causes vascular injury, arteriosclerosis and venous thrombosis [1]. Numerous studies, both retrospective and prospective, have shown that mildly elevated plasma tHcy concentration is associated with increased risk of myocardial infarction and coronary atherosclerosis [2–16]. It also seems to be a strong predictor of mortality in patients with coronary artery disease (CAD) [17,18]. The association between modestly increased plasma tHcy concentration and other atherosclerotic and thromboembolic cardiovascular disease is also well documented [19–21]. However, there are also negative reports [22–26] and it is still unclear whether mild hyperhomocysteinemia is causally linked to the development of cardiovascular disease. In most of the above mentioned studies both men and women participated, but women were often in a minority. Furthermore, coronary angiography was often not performed, adding uncertainty to the diagnosis of CAD, considering that 20–40% of women with chest pain of typical angina character do not have signs of coronary atherosclerosis at coronary angiography [27]. Only a few of the studies regarding the association of plasma tHcy and CAD in women involve patients, who were catheterized [7,11,14,16,17,25,26].
2. Subjects and methods

2.1. Patients

During a 2 year period all postmenopausal women (≥12 months since last menstruation) with a history of unstable CAD entering the coronary care unit were requested to participate in the study if they fulfilled the diagnostic criteria. These were new or increasing angina during the previous 2 months or ongoing chest pain suggesting ischemia, in conjunction with transient or persistent ST-depression and/or T-wave inversion of at least 0.1 mV in at least two adjacent leads. Most of the participants were part of the multicenter Fragmin during Instability in Coronary Artery Disease (FRISC) study [35]. Exclusion criteria were left bundle branch block, left ventricular hypertrophy or pacemaker rhythm in resting ECG, suspected myocarditis or endocarditis, cardiomyopathy, hemodynamically important aortic valve disease, planned percutaneous transluminal coronary angioplasty, planned or previously performed coronary artery bypass grafting, systolic blood pressure <90 mm Hg, fever and diseases with a poor prognosis e.g. malignant disease.

Patients who were stabilised on conventional medical therapy and had no signs of severe ischemia on a predischarge exercise test were rescheduled for coronary angiography within 2 months after discharge. Otherwise this was done in the acute phase.

An age-matched control group randomly selected from the population register was also examined. They had to be anamnestically and clinically healthy with normal routine laboratory tests, normal ECG and to be taking no medications. We did, however, consider it unethical to perform a coronary angiography on these healthy subjects.

2.2. Blood samples

Blood samples for the measurement of myocardial enzymes according to local routines (troponine-T, creatine kinase MB, creatine kinase B or creatine kinase) were obtained on admission and every 6th hour until the maximum level was passed with a minimum of three samples.

Blood samples for the analysis of plasma tHcy (μmol/l), serum folate (nmol/l), vitamin B12 (pmol/l) and serum creatinine (μmol/l) were obtained within 24 h after admission in the fasting state and after 15 min recumbency. For amino acid measurements venous blood was collected in evacuated tubes containing EDTA, immediately placed on ice, centrifuged within 15 min at 3000 × g for 5 min at −4°C, and the supernatants were stored frozen at −70°C until analysis. Plasma tHcy was measured with the method of Andersson [36]. Blood samples for analysis of serum folate, serum vitamin B12, and serum creatinine were sent to the hospital biochemical laboratory for routine analysis.

2.3. Coronary angiography

Selective coronary angiography with multiple projections was performed by a percutaneous transfemoral approach on days 50–70 after inclusion. The angiograms were evaluated visually by two independent experienced angiographers, neither of whom had any knowledge of the patient nor of each other’s judgement. The patients were subdivided according to findings at coronary angiography, i.e. normal coronary vessels, non-significant coronary atherosclerosis (<50% narrowing of the luminal diameter) and significant coronary stenosis (≥50% luminal reduction in a major epicardial vessel).
2.4. Statistics

Results are given as means with 95% confidence intervals unless otherwise indicated. Normally distributed data were tested using unpaired Student’s t-test. For non-parametric data we used Mann–Whitney U test. As the distribution of plasma tHcy was skewed to the right we present logarithmic transformed values as well. Multiple comparison was tested using the Bonferroni test. Correlation was tested by using a bivariate Spearman’s nonparametric rank correlation test. Significance of trend was evaluated using linear regression.

The association of plasma tHcy and unstable CAD was evaluated in a logistic regression model by calculating odds ratios and 95% confidence intervals for the whole patient group versus the control group, for patients with coronary atherosclerosis versus the control group and for patients with significant coronary stenosis versus the control group using quintiles for controls as reference. Furthermore, odds ratios were estimated with plasma tHcy as a continuous variable for all three groups. In the logistic regression model we first adjusted for the classical risk factors: Age (continuous), current smoking (categorical), hyperlipidemia (categorical), hypertension (categorical), diabetes mellitus (categorical), and body-mass index (continuous). In a second model we added the serum values (continuous) of folate, vitamin B12 and creatinine.

ROC analyses and plotting were performed with a β version of the program (Rockit 0.9.1B). The ROC plot gives the full spectrum of sensitivity/specificity pairs, corresponding to all of the possible decision levels, obtainable for a test in a particular clinical application. The area under the ROC curve varies from 1.0, which corresponds to perfect discrimination (upper left corner) to 0.5 where no discrimination exists. Swets [37] has suggested the following guidelines for interpreting the area: 0.5–0.7, rather low accuracy; 0.7–0.9, accuracy useful for some purposes; and greater than 0.9, rather high accuracy. For tHcy and its covariates we calculated percentiles for all the participants in the study and for the patient group, respectively. We used the former percentiles to calculate sensitivity/specificity pairs for the patient group versus the control group regarding unstable CAD or not. Likewise, the latter percentiles were used to calculate sensitivity/specificity pairs for patients with normal coronary arteries versus patients with coronary stenosis regarding significant coronary stenosis or not. A two-sided z-test was used to compare the areas under the ROC curves and areas are given with 95% confidence intervals.

Statistical significance was set at \( P < 0.05 \). All statistical analyses were performed by a computer using the SPSS system (Statistical Package for the Social Sciences INC., Chicago, USA, 1990).

The study was approved by the local scientific ethics committee. All procedures followed the Helsinki Declaration.

3. Results

3.1. Characteristics of study population

One hundred and fifty seven patients and 101 healthy controls were included in the study. The results at coronary angiography and baseline characteristics are given in Table 1. Women with angina-like chest pain and normal coronary vessels were significantly younger than the rest of the study population. Serum myocardial enzyme level above reference value was found in 43%, and more frequently in patients with significant stenosis than in patients with normal vessels (50 vs. 24%, \( P < 0.05 \)). No patient developed heart failure. At the time of blood sampling 38% of the patients were taking betablockers, 23% calcium antagonists, 22% long-acting nitroglycerin, 20% aspirin, 17% diuretics, 6% ACE-inhibitors, and 5% digoxin. Furthermore, 37% received low-molecular-weight heparin and 5% intravenous nitroglycerin. Sixteen per cent in the patient group and 14% in the control group were taking hormone replacement therapy.

All controls performed an exercise test, which was performed as previously described [38]. Seventeen of the controls had ST-depression \( \geq 0.1 \) mV during exercise. Apart from a lower mean body-mass-index (23.1 (21.7–24.5) vs. 25.1 (24.2–26.0), \( P < 0.05 \)), these women did not differ from the rest of the control group.

3.2. Plasma tHcy and covariates in patients and control subjects

Fig. 1 shows the distribution pattern of plasma tHcy in the patient group according to quintiles for the control group. Table 2 presents plasma tHcy, serum folate, serum vitamin B12 and serum creatinine in the different patient subgroups. In both the patient and control group there was a strong positive correlation between plasma tHcy concentration and serum creatinine concentration \( (r = 0.32, P < 0.001) \) and age \( (r = 0.18, P < 0.01) \), and a strong negative correlation with serum concentrations of folate \( (r = -0.33, P < 0.001) \) and vitamin B12 \( (r = -0.24, P < 0.001) \). Plasma tHcy concentrations are therefore presented unadjusted as well as adjusted for these four covariates. As Fig. 1 shows, the distribution of plasma tHcy is skewed and we therefore present logarithmic transformed values as well. Mean concentrations for plasma tHcy, serum folate and vitamin B12 were similar in patients and control subjects, whereas serum creatinine was higher in the patient group. After adjustment for these three
covariates and age, there was still no difference in plasma tHcy concentration between patients and control subjects. A comparison of the logarithmic transformed values did not show any significant differences. There was no difference in mean plasma tHcy in control subjects with or without ST-depression at exercise test (11.9 (10.5–13.3) vs. 12.3 (11.2–13.5)).

Apart from age, mean plasma tHcy concentration showed no correlation to the variables in Table 1, or to raised coronary enzyme level on admission, or to current medication.

Table 1
Baseline characteristics in 157 women with unstable CAD and in 101 healthy control persons (mean (95% CI) unless otherwise indicated*)

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 101) (A)</th>
<th>All patients (n = 157) (B)</th>
<th>Normal vessels (n = 25) (C)</th>
<th>Coronary stenosis &lt;50% (n = 23) (D)</th>
<th>Coronary stenosis ≥50% (n = 109) (E)</th>
<th>Coronary athero (n = 132) (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66.0 (64.6;67.4)</td>
<td>66.1 (65.0;67.1)</td>
<td>60.5 (57.7;63.3)**</td>
<td>67.0 (64.0;69.9)</td>
<td>67.2 (66.0;68.3)</td>
<td>67.1 (66.0;68.2)</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>7*</td>
<td>12</td>
<td>22</td>
<td>20</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Body-mass-index</td>
<td>24.7 (24.0;25.4)*</td>
<td>26.6 (26.0;27.3)</td>
<td>25.6 (24.3;26.8)</td>
<td>26.0 (24.7;27.3)</td>
<td>27.0 (26.2;27.9)</td>
<td>26.8 (26.1;27.6)</td>
</tr>
<tr>
<td>(kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist-hip-ratio</td>
<td>0.81 (0.80;0.82)*</td>
<td>0.86 (0.84;0.87)</td>
<td>0.82 (0.79;0.85)</td>
<td>0.86 (0.82;0.89)</td>
<td>0.87 (0.84;0.89)</td>
<td>0.87 (0.85;0.89)</td>
</tr>
<tr>
<td>Oophorectomy (%)</td>
<td>7</td>
<td>11</td>
<td>20</td>
<td>9</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>-</td>
<td>12</td>
<td>0***</td>
<td>9</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Previous heart failure (%)</td>
<td>-</td>
<td>3</td>
<td>0</td>
<td>13****</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>-</td>
<td>36</td>
<td>32</td>
<td>30</td>
<td>39</td>
<td>37</td>
</tr>
<tr>
<td>Hyperlipidemia (%)</td>
<td>-</td>
<td>6</td>
<td>0</td>
<td>9</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>PAD/CVI (%)</td>
<td>-</td>
<td>6</td>
<td>0</td>
<td>4</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Previous MI (%)</td>
<td>-</td>
<td>17</td>
<td>4***</td>
<td>9</td>
<td>22</td>
<td>20</td>
</tr>
</tbody>
</table>

* Normal vessels = Angina with normal coronary vessels. Coronary athero = Patients with non-significant and significant coronary stenosis pooled together. Hyperlipidemia = pharmacologically treated hyperlipidemia. PAD/CVI = peripheral arterial disease and/or cerebro-vascular incidence. MI = myocardial infarction. P<0.05:
* = A versus B, E and F;
** = C versus A, D, E and F;
*** = C versus E and F;
**** = D versus C, E and F.

Fig. 1. Plasma tHcy in 157 postmenopausal women with unstable CAD according to quintiles for 101 controls.
Table 2
Plasma tHcy, serum folate, serum vitamin B12 and serum creatinine in patients and healthy control subjects (mean (95% CI))

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 101) (A)</th>
<th>All patients (n = 157) (B)</th>
<th>Normal vessels (n = 25) (C)</th>
<th>Coronary stenosis &lt;50% (n = 23) (D)</th>
<th>Coronary stenosis ≥50% (n = 109) (E)</th>
<th>Coronary athero (n = 132) (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine (µmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homocysteine adj.</td>
<td>12.5 (11.6;13.5)</td>
<td></td>
<td>13.1 (12.3;13.8)</td>
<td>12.0 (10.8;13.2)</td>
<td>12.7 (11.5;14.0)</td>
<td>13.4 (12.4;14.4)</td>
</tr>
<tr>
<td>Log-transformed tHcy</td>
<td>2.48 (2.42;2.54)</td>
<td></td>
<td>2.53 (2.48;2.57)</td>
<td>2.46 (2.36;2.55)</td>
<td>2.52 (2.42;2.62)</td>
<td>2.54 (2.49;2.60)</td>
</tr>
<tr>
<td>Folate (nmol/l)</td>
<td>17.7 (16.0;19.5)</td>
<td></td>
<td>16.2 (14.7;17.7)</td>
<td>15.4 (11.1;19.6)</td>
<td>18.7 (13.8;23.6)</td>
<td>15.9 (14.2;17.6)</td>
</tr>
<tr>
<td>Vitamin B12 (pmol/l)</td>
<td>313 (287;340)</td>
<td></td>
<td>297 (270;323)</td>
<td>318 (239;397)</td>
<td>284 (229;340)</td>
<td>295 (262;327)</td>
</tr>
<tr>
<td>Creatinine (mmol/l)</td>
<td>79 (77;81)*</td>
<td>83 (80;85)</td>
<td>84 (79;89)</td>
<td>80 (77;87)</td>
<td>83 (80;86)</td>
<td>82 (79;85)</td>
</tr>
</tbody>
</table>

* Normal vessels = angina with normal coronary vessels. Coronary athero = patients with non-significant and significant coronary stenosis pooled together. Adj. = values adjusted for the four main covariates (serum folate, serum vitamin B12, serum creatinine and age). Log-transformed tHcy = logarithmic transformed values for plasma tHcy. There were no significant differences between the different patient groups regarding plasma tHcy. P < 0.05:
* = A versus E.

Table 3
Odds ratios (95% CI) of CAD by different percentiles of plasma tHcy for healthy control subjects.

<table>
<thead>
<tr>
<th>Percentile</th>
<th>20%</th>
<th>40%</th>
<th>60%</th>
<th>80%</th>
<th>90%</th>
<th>They cont.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy (µmol/l)</td>
<td>9.24</td>
<td>10.78</td>
<td>12.12</td>
<td>14.86</td>
<td>17.86</td>
<td></td>
</tr>
</tbody>
</table>

Odds ratios all patients
Model 1† | 0.77 (0.49;1.19) | 0.87 (0.63;1.21) | 0.84 (0.61;1.14) | 0.97 (0.66;1.43) | 3.41 (1.09;10.67) | 1.03 (0.95;1.11) |
Model 2‡ | 0.84 (0.52;1.34) | 0.94 (0.66;1.34) | 0.88 (0.63;1.24) | 1.05 (0.70;1.58) | 3.53 (1.15;10.78) | 1.05 (0.96;1.15) |

Odds ratios patients with atherosclerosis
Model 1† | 0.80 (0.49;1.30) | 0.82 (0.58;1.18) | 0.81 (0.58;1.13) | 1.03 (0.68;1.56) | 3.24 (0.98;10.68) | 1.02 (0.94;1.11) |
Model 2‡ | 0.87 (0.52;1.45) | 0.87 (0.59;1.29) | 0.84 (0.58;1.20) | 1.09 (0.69;1.70) | 3.34 (1.03;10.88) | 1.05 (0.95;1.15) |

Odds ratios patients with significant stenosis
Model 1† | 0.85 (0.51;1.41) | 0.77 (0.53;1.14) | 0.74 (0.51;1.07) | 1.00 (0.64;1.55) | 2.83 (0.87;9.22) | 1.01 (0.93;1.10) |
Model 2‡ | 0.99 (0.57;1.71) | 0.86 (0.57;1.30) | 0.80 (0.55;1.17) | 1.11 (0.69;1.78) | 2.80 (0.90;8.76) | 1.04 (0.94;1.15) |

† tHcy cont. = plasma tHcy as a continuous variable.
‡ Model 1 = adjusted for age, current smoking, hyperlipidemia, hypertension, diabetes mellitus and body-mass index.
§ Model 2 = model 1 + adjustment for serum values of folate, vitamin B12 and creatinine.

3.3. Plasma tHcy, coronary atherosclerosis and risk of CAD

Mean plasma tHcy concentration tended to increase with an increasing degree of coronary atherosclerosis, i.e. the number of vessels involved. The increase was, however, not significant when analyzing for significance of trend (P = 0.17), and the trend further weakened after adjustments for age and values of folate, vitamin B12 and creatinine in serum.

Results from our logistic regression model, as described under statistics, are presented in Table 3. We saw a tendency to significance only for the highest (>90%) percentile. Otherwise odds ratios were just around unity for all percentiles, with confidence intervals including the null value and no obvious graded effect.

3.4. ROC analyses

The areas under the ROC curves are presented in Table 4, and varied from 0.43 to 0.77. Serum creatinine and plasma tHcy were better than chance to discriminate patients with unstable CAD from controls, but with a rather low accuracy according to the guidelines suggested by Swets [37]. For the ability to discriminate patients with normal vessels from patients with significant coronary stenosis, age was outstanding with a ROC area of 0.77, which was significantly higher than all the other variables, Table 4 and Fig. 2. However, age did not represent high diagnostic accuracy. For example, at a sensitivity of 0.8 the false-positive fraction was 0.39 (specificity 61%), and at 90% sensitivity the false-positive fraction was 0.53 (specificity 47%).
Table 4  
The areas under the ROC curves (95% C.I.) for diagnosing unstable CAD and significant stenosis, respectively

<table>
<thead>
<tr>
<th></th>
<th>All patients with unstable CAD</th>
<th>Patients with significant stenosis</th>
<th>P versus age*a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine</td>
<td>0.56 (0.49;0.63)</td>
<td>0.58 (0.46;0.69)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Folate</td>
<td>0.43 (0.36;0.50)</td>
<td>0.53 (0.40;0.65)</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>0.44 (0.37;0.51)</td>
<td>0.46 (0.34;0.58)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.57 (0.50;0.64)</td>
<td>0.45 (0.33;0.56)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age</td>
<td>0.50 (0.43;0.58)b</td>
<td>0.77 (0.64;0.87)</td>
<td></td>
</tr>
</tbody>
</table>

*a Statistics: only for patients with significant stenosis.

b Expected ROC area as patients and controls were age-matched.

4. Discussion

The results of the present study of plasma tHcy in female patients with signs and symptoms of unstable CAD and female age-matched healthy control subjects, do not support the hypothesis that mild hyperhomocysteinemia is a risk factor for CAD in postmenopausal women. There was no difference in plasma tHcy concentration between patients and controls, and no significant difference between patients with or without coronary atherosclerosis. In the highest percentile for plasma tHcy concentration (≥ 90th percentile in controls) we saw a tendency to increased risk for unstable CAD and coronary atherosclerosis. ROC analyses showed that none of the evaluated variables were useful for discriminating women with unstable CAD from the control group. Age was by far the best variable regarding the ability to discriminate patients with normal coronary vessels from patients with significant coronary stenosis, although it did not have a particularly high discriminatory power.

A question to ask is whether blood sampling during the acute phase of unstable CAD has influenced the present results. Two studies have found lower plasma tHcy concentration in the acute phase of myocardial infarction than at follow-up [39,40]. We do not have follow-up analyses. However, plasma tHcy concentrations in those 43% of our patients who showed an increase in their myocardial enzyme level, suggesting possible myocardial damage, were not lower than in those with normal enzyme levels. Therefore, we find it reasonable to assume that the acute phase of unstable CAD has not influenced the present results.

As expected, serum folate and serum vitamin B12 were negatively correlated and age and serum creatinine positively correlated, to plasma tHcy concentration. These four variables are well-known determinants of plasma tHcy [1,41]. A tendency to increasing plasma tHcy concentration with increasing degree of coronary atherosclerosis was attenuated after adjustment for these correlates.

More than 70% of plasma homocysteine is probably metabolized by the kidney [42]. Patients with atherosclerosis are likely to have a higher serum creatinine because of concomitant nephrosclerosis. This, and the well-known age-related decline in renal function, makes it important to adjust the risk-estimate associated with tHcy for the levels of serum creatinine, although this measure is a poor indicator of early renal decline. We also adjusted for serum levels of folate and vitamin B12, which some may not find relevant, arguing that the hypothesis of homocysteine involves low values of these two vitamins. However, in more studies there are no differences between patients and controls regarding one or both of these vitamins in spite of differences in plasma tHcy [5,10,14,16,23,40]. Unfortunately, in most of the below mentioned studies there is scarce information about the covariates, or the authors have not adjusted for these in spite of differences between the groups studied.

Compared to other studies on this subject our study falls in the middle regarding the size of the population studied. However, it is to our knowledge the largest study of angiographically examined female patients. Catheterization was not done in the control group, and we cannot exclude that some of these clinically healthy...
women had subclinical coronary atherosclerosis. Seventeen of the control women had ST-depression $\geq 0.1$ mV without other signs or symptoms of CAD during exercise. We do not know if the ST-depression represents CAD in these healthy women. However, other investigators have shown a low prevalence, $\leq 10\%$, of CAD in totally asymptomatic women with ST-depression in this age group [43].

Previous studies including female patients, who were catheterized, have shown diverging results. Robinson found in 103 women an increasing risk of having CAD with increasing plasma tHcy irrespective of age and gender and with no threshold effect [7], and Nygård found plasma tHcy to be a strong predictor of mortality in 109 women [17]. The latter study is difficult to interpret, because it includes patients with a marked difference in risk, from patients with stable angina and no previous myocardial infarction to patients with previous coronary bypass-operation and a new myocardial infarction. Furthermore, and rather surprisingly, the authors did not find an effect of coronary revascularisation. Three small studies including up to 27 women showed plasma tHcy to be a risk factor for coronary atherosclerosis [11,14,16], whereas two other small studies, including 36 and 58 catheterized female patients, respectively, did not show homocysteine to be related to CAD [25,26].

In women not catheterized there are positive [5,8,10,12,13,15,19] as well as negative studies [22,24], regarding plasma tHcy and cardiovascular disease. More of these studies include rather young patients [5,8,10,22,24], where one might expect less atherosclerotic and more thrombotic disease, and the results therefore may be difficult to interpret.

As confirmed in the present study, serum folate is an important determinant of plasma tHcy concentration [44]. Recently, a common mutation in the gene of methyltetrahydrofolate reductase was identified [45]. 10% of the caucasian population are mutant homozygotes and have reduced enzyme activity leading to an average of 2.5 $\mu$mol/l or 25% higher plasma tHcy concentration than normal homozygotes [46–50]. Consequently, the mutation was strongly suspected to be a common genetic risk factor for cardiovascular disease, which rapidly led to several studies [48–51]. A recently published metaanalysis of 23 of these studies showed that the mutation, although it is a major cause of mild hyperhomocysteinemia, does not increase cardiovascular risk [51]. Furthermore, a study on this mutation in relation to longevity showed no relation to premature death [52].

Our study does not settle the debate on whether or not mild hyperhomocysteinemia is an independent risk factor for cardiovascular disease. However, the strength of our study is our very well-defined and homogeneous patient population, with a high prevalence of CAD verified at coronary angiography. Our control group was age-matched and was clinically healthy and with a very low probability of CAD. We therefore find it reasonable to expect that we would have detected a difference between these two groups of diseased and healthy individuals if one such had existed.

In conclusion, our study does not support the hypothesis that mild hyperhomocysteinemia is a risk factor for unstable CAD in postmenopausal women. We suspect that the trend towards higher plasma tHcy with increasing degree of coronary atherosclerosis may be a marker, but not a cause of the disease, although our study was not designed to differentiate between this. In future studies adjustments should be considered for the four main covariates regarding plasma tHcy, namely age and serum values of folate, vitamin B12 and creatinine, as minor variations in these strongly influence the statistics, as shown in this study.

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