Hyperhomocystinemia

A Risk Factor or a Consequence of Coronary Heart Disease?

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Background: Mild hyperhomocystinemia has been suggested as an indicator of an increased risk of cardiovascular disease.

Objective: To examine whether serum homocysteine concentration is a predictor of coronary heart disease (CHD) events.

Methods: A case-control study, nested in a population-based cohort study was used. During a follow-up of 13 years, 166 major coronary events (death from CHD or nonfatal myocardial infarction) occurred in men with evidence of heart disease at baseline and 272 events in men without a history of heart disease. Two controls per case were selected by individual matching.

Results: Among men with known heart disease at baseline, the relative risk (95% confidence interval) of CHD events adjusted for age, smoking, hypertension, diabetes mellitus, serum cholesterol level, body mass index, and alcohol consumption was 2.23 (95% confidence interval, 1.03-4.85) in the highest serum homocysteine quintile compared with the lowest quintile. Among the men free of heart disease at baseline, the corresponding relative risk was 0.90 (95% confidence interval, 0.51-1.60).

Conclusions: This prospective study does not support the hypothesis that a high concentration of serum homocysteine is a risk factor for coronary events in a population free of heart disease. However, it does suggest that mild hyperhomocystinemia predicts secondary coronary events in men with heart disease, possibly as a consequence of atherosclerotic changes.

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ELEVATED plasma concentration of homocysteine has been suggested to be a new important risk factor of atherosclerotic vascular disease amenable to preventive actions.1-3 Experimental evidence suggests that an increased concentration of homocysteine may result in vascular changes through several mechanisms.4-6 Homocysteine has been shown to impair vascular endothelial cell function and even induce cell damage. In addition, homocysteine is a stimulator of smooth muscle cell proliferation. It may also induce oxidation of low-density lipoproteins. Thrombogenesis is enhanced by a high concentration of homocysteine, increased adherence of platelets, inhibition of protein C activation, expression of thrombomodulin, and decreased activity of tissue-type plasminogen activator. Several case-control studies have provided consistent evidence showing higher serum homocysteine concentrations in patients with various atherosclerotic diseases compared with healthy control subjects.1,5,7,8 Accordingly, the homocysteine risk factor hypothesis has won large acceptance and several authors5,9-12 have argued that screening and preventive measures should be undertaken at least in persons with known atherosclerotic disease. Folic acid is known to be an effective factor in reducing elevated levels of serum homocysteine, and several intervention studies have been initiated to investigate the effect of supplementation of the vitamin on hyperhomocystinemia risk and in secondary prevention among individuals with known atherosclerotic diseases.13,14

The fact remains that the hypothesis has been strongly based on biological mechanisms mostly demonstrated by in vitro studies and that the human evidence mainly comes from case-control studies whereas population-based prospective cohort studies have given conflicting results. Several cohort studies showed a significantly elevated risk of coronary heart disease (CHD) at higher serum homocysteine levels.15-22 Other stud-
SUBJECTS, MATERIALS, AND METHODS

A total of 3471 men, aged between 45 and 64 years, participated in the Mobile Clinic Health Examination Survey that was carried out in various regions of Finland from January 1, 1973 through December 31, 1976. The individuals participated with the understanding that these data would be used in scientific research. A self-administered questionnaire provided information about the history of diseases, medicine use, recreational physical activity, alcohol consumption, and smoking habits. The answers to this questionnaire were checked and, if necessary, specially trained nurses assisted subjects in its completion. Casual blood pressure was registered, and the subjects were classified into 4 hypertension categories based on their systolic and diastolic blood pressures and their use of antihypertensive drugs. Body height and weight were measured and the body mass index (calculated as weight in kilograms divided by the square of height in meters) was computed. Serum cholesterol concentrations were determined from serum samples after 13 weeks of storage at −20°C with an autoanalyzer modification of the Burchard-Liebermann reaction. Patients with heart disease were identified based on disease history. Patients with diabetes mellitus were identified on the basis of disease history, use of antidiabetic medication, or the results of an oral glucose tolerance test. A history of heart disease was obtained using specific questions: Have you had, according to a physician’s diagnosis, myocardial infarction, angina pectoris, heart failure, or valvular or congenital heart disease? Of the 3471 men, 884 reported a history of heart disease.

Nonfatal cases of myocardial infarction (International Classification of Diseases, Eighth Revision code 410) were identified by linking the study population to the nationwide hospital discharge register using a unique personal identification number. The fatal cases of CHD (International Classification of Diseases, Eighth Revision codes 410-414) were identified from death certificates that were obtained for all the deceased from Statistics Finland, Helsinki. For persons with several CHD events, the first one was registered. The follow-up covered cases occurring between the baseline examination and the end of 1985. During this follow-up period 166 major CHD events (coronary deaths or myocardial infarction) occurred among the men with heart disease and 272 such events among those free of heart disease at baseline.

A nested case-control design was adopted to study the ability of the serum homocysteine level to predict major CHD events. Two controls per case were selected by individual matching using age, municipality, and presence of heart disease at baseline as matching variables. The ages were matched by nearest available matching. Matching for municipality also controlled for the time of the baseline examination and for the duration of serum sample storage. Serum samples for some of the controls were for different reasons unavailable and, thus, the final numbers of controls were 311 and 524 for those cases with and without heart disease at baseline, respectively.

The serum samples were stored at −20°C until 1996 when they were used in the present study. The serum samples for each case and individually matched controls were analyzed simultaneously in random order independently of case-control status of which the laboratory personnel were unaware. The levels of total serum homocysteine, cysteine, and cysteinylglycine were determined by a modification of the high-pressure liquid chromatographic method described by Ubbink et al. Our mobile phase was modified to consist of 0.37-mol/L acetate and 0.5% methanol, pH 4.15. The peak heights were calibrated using a secondary serum standard. The precision between each series for an in-house serum pool was 3.3% at a level of 6.5 µmol/L. The accuracy was verified by participating in an interlaboratory quality control scheme in which the bias was null for serum at 9.5 µmol/L and +1% for serum at 38.1 µmol/L.

The conditional logistic model was used to estimate the relative risks (as odds ratios) of CHD events between quintiles (based on the distribution among all controls) of serum homocysteine. The effects of potential confounding factors were adjusted for by including them in the model. Test for trends was performed by including homocysteine concentrations as a continuous variable in the model. Age-adjusted mean levels of potential confounding factors at baseline in quintiles of serum homocysteine were estimated using the general linear model.

RESULTS

In men free of heart disease at baseline, differences were observed between subjects with incident CHD events and their controls in several (smoking, hypertension, diabetes mellitus, body mass index, serum cholesterol level, and serum triglyceride levels), but not all, (leisure time physical activity and alcohol consumption) cardiovascular risk factors (Table 1). No differences were observed for the levels of serum homocysteine, cysteine, or cysteinylglycine. The differences in cardiovascular risk factors between cases and controls in men with known heart disease at baseline were similar. In that subpopulation, however, the mean serum homocysteine concentration was significantly (9%) higher in cases than in controls. The age-adjusted serum homocysteine level among future CHD events with known heart disease at baseline...
Serum homocysteine concentration increased significantly with age (Table 2). It was also directly proportional to the prevalence of arterial hypertension and to the level of serum triglycerides. Furthermore, serum homocysteine concentration was strongly associated with the serum cysteine level and weakly associated with the serum triglyceride level. Furthermore, serum homocysteine, 7.397 to convert to micromoles per liter.

No association between serum homocysteine concentration and the incidence of major CHD events was observed in men originally free of heart disease (Table 3). The relative risk between individuals in the highest and lowest quintiles of serum homocysteine was 1.00 (95% confidence interval [CI], 0.61-1.63). A similar comparison between the highest and lowest deciles gave a corresponding value of 1.11 (95% CI, 0.55-2.25). In men known to have heart disease at baseline, however, there was a positive association between the serum homocysteine level and the incidence of major CHD events. The relative risk of such an event between the highest and lowest homocysteine quintiles was 2.15 (95% CI, 1.10-4.22). Adjustment for the known risk factors of CHD, ie, smoking, hypertension, diabetes mellitus, serum cholesterol level, body mass index, and alcohol consumption, did not notably alter the result (Table 3).

An examination of possible effect-modification by sex, age, body mass index, smoking, hypertension, diabetes mellitus, and high body iron stores (ferritin) on the association between the homocysteine level and the risk of major CHD events generally revealed no significant interactions (data not shown). However, in men suffering from heart disease at baseline, there was a strong posi-

### Table 1. Mean Values of Different Variables in Patients With Coronary Heart Disease Events and Control Subjects

<table>
<thead>
<tr>
<th>Baseline Variable</th>
<th>Case Mean (SD)</th>
<th>Control Mean (SD)</th>
<th>P Value for Difference</th>
<th>Case Mean (SD)</th>
<th>Control Mean (SD)</th>
<th>P Value for Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>54.4 (5.6)</td>
<td>54.2 (5.6)</td>
<td>.03</td>
<td>55.8 (5.4)</td>
<td>55.6 (5.4)</td>
<td>.12</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>58.0</td>
<td>36.1</td>
<td>&lt;.001</td>
<td>45.5</td>
<td>33.6</td>
<td>.009</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>7.7</td>
<td>4.4</td>
<td>.07</td>
<td>15.7</td>
<td>6.3</td>
<td>.003</td>
</tr>
<tr>
<td>Leisure time physical activity, %</td>
<td>11.8</td>
<td>13.4</td>
<td>.51</td>
<td>4.2</td>
<td>9.7</td>
<td>.03</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>27.6</td>
<td>19.1</td>
<td>.007</td>
<td>37.3</td>
<td>31.0</td>
<td>.17</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.2 (3.4)</td>
<td>25.6 (2.4)</td>
<td>.02</td>
<td>26.8 (3.7)</td>
<td>26.6 (2.9)</td>
<td>.59</td>
</tr>
<tr>
<td>Alcohol consumption, g/mo</td>
<td>240 (316)</td>
<td>270 (269)</td>
<td>.21</td>
<td>193 (265)</td>
<td>248 (324)</td>
<td>.09</td>
</tr>
<tr>
<td>Serum cholesterol level, mg/dL</td>
<td>300 (57)</td>
<td>272 (35)</td>
<td>&lt;.001</td>
<td>295 (57)</td>
<td>280 (39)</td>
<td>.009</td>
</tr>
<tr>
<td>Serum triglyceride levels, mg/dL</td>
<td>160 (125)</td>
<td>127 (50)</td>
<td>&lt;.001</td>
<td>179 (112)</td>
<td>147 (50)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Serum creatinine level, mg/dL</td>
<td>1.0 (0.26)</td>
<td>1.0 (0.25)</td>
<td>.92</td>
<td>1.0 (0.19)</td>
<td>1.0 (0.15)</td>
<td>.57</td>
</tr>
<tr>
<td>Serum homocysteine level, mg/dL</td>
<td>0.146 (0.046)</td>
<td>0.152 (0.063)</td>
<td>.35</td>
<td>0.163 (0.076)</td>
<td>0.149 (0.040)</td>
<td>.03</td>
</tr>
<tr>
<td>Serum cysteine level, mg/dL</td>
<td>2.77 (0.52)</td>
<td>2.77 (0.41)</td>
<td>.94</td>
<td>2.86 (0.50)</td>
<td>2.83 (0.40)</td>
<td>.50</td>
</tr>
<tr>
<td>Serum cysteine–homocysteine ratio</td>
<td>23.3 (5.3)</td>
<td>23.6 (4.7)</td>
<td>.57</td>
<td>22.6 (5.9)</td>
<td>23.8 (4.4)</td>
<td>.02</td>
</tr>
<tr>
<td>Serum cysteine/glycine level, µmol/L</td>
<td>31.3 (6.2)</td>
<td>31.5 (4.7)</td>
<td>.62</td>
<td>32.3 (6.4)</td>
<td>32.4 (5.2)</td>
<td>.80</td>
</tr>
</tbody>
</table>

*To convert the serum cholesterol level to Système International units multiply by 0.02586 to convert to millimoles per liter; serum triglycerides, 0.01125 to convert to millimoles per liter; serum creatinine, 88.4 to convert to micromoles per liter; and cysteine, multiply by 82.67 to convert to micromoles per liter; and serum homocysteine, 7.397 to convert to micromoles per liter.

### Table 2. Mean Values* of Different Variables in Quartiles of Serum Homocysteine Among All Controls Combined

<table>
<thead>
<tr>
<th>Baseline Variable</th>
<th>Homocysteine Quartile</th>
<th>P Value for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (Lowest)</td>
<td>2</td>
</tr>
<tr>
<td>Age, y†</td>
<td>53.4</td>
<td>54.4</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>39.2</td>
<td>39.9</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>6.2</td>
<td>3.9</td>
</tr>
<tr>
<td>Leisure time physical activity, %</td>
<td>12.4</td>
<td>12.1</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>17.9</td>
<td>18.6</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.5</td>
<td>25.9</td>
</tr>
<tr>
<td>Alcohol consumption, g/d</td>
<td>258</td>
<td>256</td>
</tr>
<tr>
<td>Serum cholesterol level, mg/dL</td>
<td>271</td>
<td>273</td>
</tr>
<tr>
<td>Serum triglyceride levels, mg/dL‡</td>
<td>128</td>
<td>130</td>
</tr>
<tr>
<td>Serum creatinine level, mg/dL‡</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Serum cysteine level, mg/dL‡</td>
<td>203</td>
<td>224</td>
</tr>
<tr>
<td>Serum cysteine/glycine level, µmol/L</td>
<td>31.1</td>
<td>31.2</td>
</tr>
</tbody>
</table>

*The mean values were adjusted for age.
†There was no adjustment for age.
‡To convert the serum triglyceride level to Système International units multiply by 0.01125 to convert to millimoles per liter; serum creatinine, multiply by 88.4 to convert to micromoles per liter; and cysteine, multiply by 82.67 to convert to micromoles per liter.
tive association between the homocysteine concentration and the major CHD events in the lowest tertile of serum ferritin. In this category the relative risk of CHD events between the highest and lowest quartiles of homocysteine was 7.11 (95% CI, 2.05-24.74).

A study of the association as a function of follow-up time suggested strong associations for shorter follow-up times both in men with and without heart disease at baseline. The relative risks of major CHD events between the highest and lowest quintiles of homocysteine during the first 2 years of follow-up were 4.44 (95% CI, 1.01-19.50) and 2.38 (95% CI, 0.53-10.64), respectively, in the 2 subgroups of men.

We found that an elevated serum concentration of homocysteine predicted CHD events in men with evidence of known heart disease at the baseline examination. In men without a history of heart disease, however, homocysteine did not predict future disease. Our results do not therefore corroborate the hypothesis that homocysteine concentration is a causal factor in the pathogenesis of atherosclerosis in healthy populations. Similar negative findings were reported in another prospective population study in Finland.43 As in our study, one earlier report could not demonstrate an increased subsequent CHD event risk in men with known heart disease at baseline.26 These findings could partly be explained by the existence of a varying proportion of individuals with known or unknown atherosclerotic disease at baseline examina-

**COMMENT**

Second, the strength of association of homocysteine concentration and atherosclerosis may vary between populations. The genetic background of the Finnish population differs somewhat from that of the populations from other Western countries. Thus, some hereditary disorders common in other countries are rare in Finland, whereas many hereditary disorders occurring in Finland are rare in other countries.51,44 Homocystinuria is one of the hereditary disorders that are rare in Finland.43 As in our study, one earlier report could not find any effect of serum homocysteine concentration on the risk of atherosclerotic diseases in Finnish people free of heart disease at baseline.26 These findings could partly be explained by the rarity of genetically determined disorders in Finnish people leading to elevated levels of serum homocysteine. The strength of association may also vary from one subpopulation to another, according to single effect-modifying factors, such as sex,22 age,21 and hypertension,19 or clusters of several cardiovascular risk factors. In this study, we found no notable interactions with known cardiovascular risk factors.

Third, the serum homocysteine concentration may be associated with established risk factors and, thus, the

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**Table 3. Relative Risk of Coronary Heart Disease Events Between Quintiles of Serum Homocysteine**

<table>
<thead>
<tr>
<th>Quintile, µmol/L</th>
<th>No. of Cases</th>
<th>No. of Controls</th>
<th>No Adjustment RR 95% CI</th>
<th>Adjustment† RR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 7.9</td>
<td>60</td>
<td>102</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7.9-9.1</td>
<td>49</td>
<td>114</td>
<td>0.75 0.47-1.19</td>
<td>0.69 0.40-1.19</td>
</tr>
<tr>
<td>9.2-10.4</td>
<td>42</td>
<td>108</td>
<td>0.65 0.40-1.06</td>
<td>0.64 0.38-1.12</td>
</tr>
<tr>
<td>10.5-12.3</td>
<td>65</td>
<td>106</td>
<td>1.05 0.67-1.66</td>
<td>0.77 0.45-1.30</td>
</tr>
<tr>
<td>≥12.4</td>
<td>56</td>
<td>94</td>
<td>1.00 0.61-1.63</td>
<td>0.90 0.51-1.60</td>
</tr>
</tbody>
</table>

*RR indicates relative risk; 95% CI, 95% confidence interval.
†Adjustment was made for smoking, hypertension, diabetes, serum cholesterol, body mass index, and alcohol consumption.
observed association could be due to uncontrolled confounding factors. As in earlier studies, we found that age, hypertension, body mass index, and serum triglyceride levels were associated with the level of homocysteine. In the Caerphilly Prospective Study, in which a significantly increased risk associated with high homocysteine levels was observed, statistical significance was not reached once other risk factors were controlled for. In another British prospective study, the British United Provident Association Study, the significance of the association for serum homocysteine was reported after controlling only for systolic blood pressure and serum apolipoprotein B. In our study, as well as in another, adjustment for potential confounding factors did not appreciably alter the strength of the association.

Fourth, the lack of association may be owing to poor reliability of serum homocysteine determination. The analytical variation in our study was, however, acceptably low. The long storage of our serum samples at −20°C could potentially weaken the reliability of serum determinations. It has, however, been shown that the length of storage time apparently does not lead to alterations in homocysteine concentrations. Furthermore, all serum samples had undergone the same freeze-thaw history and were analyzed within a short time without knowledge of the case-control identification. The fact that we found an association among men with heart disease also suggests that the reliability of serum homocysteine determination was adequate.

Finally, although there are several possible mechanisms by which a high homocysteine level may promote pathogenesis of atherosclerosis, doubts of the causal role have recently been raised. Evidence has been presented that plasma homocysteine level increases after tissue damage and raised plasma levels of homocysteine promote endothelial damage and adhesion of leukocytes to the endothelial surface. A high plasma homocysteine level, thus, would be an indicator of continuing tissue damage and raised plasma levels of homocysteine rather than a primary risk factor.

CONCLUSIONS

Our prospective data demonstrating the ability of high serum concentrations of homocysteine to predict secondary CHD events in middle-aged men with heart disease do not support the hypothesis that a high homocysteine concentration is a primary causal factor in the pathogenesis of atherosclerosis, but merely suggest it to be a consequence of atherosclerotic changes.

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REFERENCES