Clinical Investigation and Reports

Plasma Total Cysteine as a Risk Factor for Vascular Disease

The European Concerted Action Project

Lina El-Khairy, MPhil; Per M. Ueland, MD; Helga Refsum, MD; Ian M. Graham, FRCPI; Stein E. Vollset, MD, DrPH

Background—Elevated plasma total homocysteine (tHcy) is a risk factor for cardiovascular disease. Although cysteine is structurally similar and metabolically linked to tHcy, its relation to the risk of cardiovascular disease has received little attention. We studied the relation between plasma total cysteine (tCys) levels and the risk of vascular disease in the coronary, cerebral, and peripheral vessels.

Methods and Results—This case-control study included 750 patients with vascular disease and 800 age- and sex-matched control subjects recruited from 19 centers in 9 European countries. Conventional risk factors for cardiovascular disease were recorded. In addition, plasma levels of tCys, tHcy, folate, B₁₂, and creatinine were measured. Overall, a U-shaped relationship was observed between tCys and risk of vascular disease. With the middle range of 250 to 275 μmol/L tCys used as the reference category, the adjusted risk of vascular disease at low (<225 μmol/L) tCys levels was 2.1 (95% CI 1.2 to 3.6), and the risk at high (>300 μmol/L) tCys levels was 1.6 (95% CI 1.1 to 2.3). Different shapes of the dose-response relationship were seen for the 3 vascular disease categories. The relation with peripheral vascular and cerebrovascular disease was U-shaped, whereas a weak positive relation was observed with coronary heart disease.

Conclusions—Our data show a significant U-shaped relationship between tCys and cardiovascular disease after adjustment for tHcy, creatinine, and other cardiovascular disease risk factors. (Circulation. 2001;103:2544–2549.)

Key Words: cardiovascular diseases • amino acids • risk factors
plasma tHcy level is an independent risk factor for cardiovascular disease. The magnitude of risk was similar to that of smoking or hyperlipidemia. Moreover, tHcy interacted with smoking and hypertension and thereby conferred a marked risk enhancement. In the present work, we investigated the relation between levels of plasma tCys and occlusive disease in the coronary, cerebral, and peripheral vessels by using data from this large case-control study.

Methods

Study Population

A total of 750 patients with coronary heart disease, cerebrovascular disease, or peripheral vascular disease (cases) and 800 control subjects (controls) were included in this study. Both cases and controls were <60 years of age, of both sexes, and were recruited from 19 centers in 9 European countries.

The exclusion and inclusion criteria have been reported in detail elsewhere. In brief, exclusion criteria included nonatherosclerotic vascular disease, cardiomyopathy, diabetes mellitus, pregnancy, systemic illness during the previous 3 months, and psychiatric illness. Patients with conditions thought to influence tHcy concentrations, such as renal or thyroid disease, anticonvulsant therapy, and recent (<3 months) exposure to nitrous oxide, were also excluded.

Cases had detailed clinical and investigational evidence of vascular disease, and 69% were recruited within 1 year of diagnosis. Controls were free of overt disease, and 69% were recruited within 1 year of diagnosis. Controls to avoid a possible effect of treatment or change in lifestyle between tCys, conventional cardiovascular disease risk factors, and creatinine. Variables examined in this study and their collection are extensively reported elsewhere.

Smokers were defined as those currently smoking any tobacco (at the time of diagnosis for cases and at the time of the methionine-loading test for controls). Subjects were divided into 5 categories: never smokers, former smokers, light smokers (1 to 9 cigarettes/d), moderate smokers (10 to 19 cigarettes/d), and heavy smokers (≥20 cigarettes/d).

Creatinine levels were grouped into 5 categories: <50, 50 to 70, 70 to 100, 100 to 120, and >120 μmol/L.

Fasting tCys values were used for the analyses and were categorized as follows: <225, 225 to 250, 250 to 275, 275 to 300, and >300. The lowest risk for vascular disease was observed in the middle category of 250 to 275 μmol/L. Therefore, this category served as the reference group. Throughout the text, intervals defining categories of tCys, tHcy, and creatinine are half-open, including the exact lower value and excluding the upper.

Biochemical Analyses

Plasma tCys and tHcy were measured by a previously described method involving reduction with sodium borohydride, derivatization with monobromobimane, high-performance liquid chromatography (HPLC) separation, and fluorescence detection. Measurements of serum lipids, folate, vitamin B12, and vitamin B6 were determined as pyridoxal 5'-phosphate, and creatinine were performed at Mime-AB, as described. Vitamin B12 and folate concentrations were measured with a radioimmunoassay technique, and pyridoxal 5'-phosphate was measured by enzymatic photometry with HPLC separation.

Statistical Methods

Linear regression analyses were performed to assess the relationship between tCys, conventional cardiovascular disease risk factors, and other possible confounders. These analyses were performed only in controls to avoid a possible effect of treatment or change in lifestyle on these relationships.

The relationship between disease and tCys was studied by use of conditional logistic regression performed for all vascular disease
Dose-response relation between tCys levels and odds ratio for all vascular disease, coronary heart disease, cerebrovascular disease, and peripheral vascular disease. Values are adjusted for age, sex, center, creatinine, smoking, diastolic blood pressure, tHcy, BMI, and cholesterol by use of generalized additive logistic regression. Solid line indicates estimated dose-response curve; shaded area, 95% CI.

Dose-response graphs between tCys and the risk of disease were performed at separate tHcy tertiles (results not shown). For coronary heart disease, there was no relation with tCys at low tHcy levels, but a positive relation existed at medium and at high tHcy concentrations. For cerebrovascular and peripheral vascular disease, low tCys was associated with increased risk at all tHcy levels.

Analyses were repeated in men and in women separately, and essentially similar results were obtained (data not shown).

**Cardiovascular Risk at Various Combinations of tCys and tHcy**

We investigated a combination variable of tCys and tHcy in relation to the vascular risk.

The lowest adjusted risk was observed in subjects having a combination of medium tCys (250 to 300 μmol/L) and low tHcy (≤12 μmol/L); we used this group as reference. The relationship between the tCys-tHcy combinations and the risk of disease differed between the 3 vascular disease categories. Notably, the combination of low tCys and low tHcy had an increased risk for cerebrovascular disease (OR 2.2, 95% CI 1.3 to 3.9) and peripheral vascular disease (OR 1.7, 95% CI 0.9 to 3.5) but not for coronary heart disease (OR 0.6, 95% CI 0.3 to 0.9). The highest risk for all disease categories was observed for the combination of low tCys and high tHcy. The odds ratios and 95% CIs for coronary heart disease, cerebrovascular disease, and peripheral vascular disease in this group were 2.3 (1.0 to 5.3),
TABLE 2. ORs (95% CI) for All Vascular Disease, Coronary Heart Disease, Cerebral Vascular Disease, and Peripheral Vascular Disease by Plasma tCys Level*

<table>
<thead>
<tr>
<th>Plasma tCys, µmol/L</th>
<th>Controls, n</th>
<th>All Vascular Disease</th>
<th>Coronary Heart Disease</th>
<th>Cerebral Vascular Disease</th>
<th>Peripheral Vascular Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>OR (95% CI)</td>
<td>n</td>
<td>OR (95% CI)</td>
<td>n</td>
</tr>
<tr>
<td>≤225</td>
<td>52</td>
<td>2.1 (1.2–3.6)</td>
<td>13</td>
<td>0.7 (0.3–1.6)</td>
<td>24</td>
</tr>
<tr>
<td>225–250</td>
<td>163</td>
<td>1.2 (0.8–1.8)</td>
<td>40</td>
<td>0.8 (0.5–1.2)</td>
<td>46</td>
</tr>
<tr>
<td>250–275 (reference)</td>
<td>246</td>
<td>1.0</td>
<td>95</td>
<td>1.0</td>
<td>42</td>
</tr>
<tr>
<td>275–300</td>
<td>178</td>
<td>1.6 (1.2–2.3)</td>
<td>111</td>
<td>1.3 (0.9–2.0)</td>
<td>55</td>
</tr>
<tr>
<td>&gt;300</td>
<td>6</td>
<td>1.6 (1.1–2.3)</td>
<td>124</td>
<td>1.3 (0.9–2.0)</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>1.6 (1.1–2.3)</td>
<td>124</td>
<td>1.3 (0.9–2.0)</td>
<td>44</td>
</tr>
</tbody>
</table>

*Adjusted for sex, age, center, diastolic blood pressure, cholesterol, smoking, creatinine, BMI, tHcy, and triglycerides.
†Number of cases.

Discussion

In this multicenter case-control study, we analyzed the relationship between plasma tCys and the risk of cardiovascular disease in the coronary, cerebral, and peripheral arteries. Our results showed significant relationships between tCys and vascular disease. Different dose-response relationships for the 3 types of vascular disease were observed, however. The relationship with peripheral and cerebrovascular disease was U-shaped, with lowest risk in subjects with intermediate levels (250 to 275 µmol/L) of tCys, whereas a weak positive relation was observed with coronary heart disease. The association between tCys and cerebrovascular and peripheral vascular disease persisted after adjustment for conventional cardiovascular disease risk factors, tHcy, creatinine, and other potential confounders (Figure). The relationship between tCys and coronary heart disease, however, was statistically insignificant after adjustment for tHcy.

This case-control study was originally designed to evaluate the relation between tHcy and the risk of vascular disease. The availability of data on plasma levels of tCys allowed us to evaluate the relation between tCys and the risk of vascular disease in this large multicenter study. The major strength of our study is the large number of cases that allowed us to study the tCys-disease relationship separately for coronary heart disease, cerebrovascular disease, and peripheral vascular disease. This also allowed us to make more precise estimates of the strength and shape of the tCys-disease relationships. Because the blood samples in cases were drawn after the disease episode, however, we cannot rule out the possibility that tCys levels might be influenced by the disease itself. There is also the possibility that medication or change in lifestyle and dietary habits might have influenced the levels of tCys in cases. We have previously demonstrated that tCys is positively related to cholesterol, diastolic blood pressure, and BMI, which is essentially confirmed in the present study. Conceivably, cholesterol-reducing or antihypertensive therapy or weight reduction in cases may weaken rather than increase the difference in tCys between cases and controls.

The relation between tCys and cardiovascular disease was evaluated for different combinations of tCys and tHcy concentrations. Subjects with low tCys levels and high tHcy had the highest ORs for disease in all 3 vascular disease categories. The elevation in tHcy in this group may be due to low vitamin B status. In line with this, we observed a lower mean concentration of folate in this group (Table 3). Subjects...
with high tCys and high tHcy levels also had a significant increase in the risk of disease in the 3 vascular disease categories. The associated increase in serum creatinine (Table 3) suggests that elevation of both tCys and tHcy is due to an impaired renal function.28

Renal failure has consistently been shown to cause increased levels of both tHcy and tCys,29 and it is associated with high cardiovascular mortality and morbidity.30 Our finding of increased risk of cardiovascular disease and elevated serum creatinine in subjects with the combination of high tCys and tHcy (Table 3) are in accordance with these published data. One may speculate whether elevated tCys affects the development of vascular lesions in patients with renal failure.

Subjects with low tCys and low tHcy had an odds ratio >1 for having cerebrovascular and peripheral vascular disease but not coronary heart disease. The metabolic processes responsible for this aminothiol profile and the possible mechanisms behind increased risk are not readily apparent. Low tCys might be a marker of low glutathione, however, which recently was associated with increased risk of coronary heart disease.31 Cysteine is the limiting amino acid for the biosynthesis of glutathione,32 and this explained a possible link between these 2 thiol compounds.

The relation between tCys and cardiovascular disease was also evaluated at low, medium, and high tHcy levels by use of dose-response curves. For coronary heart disease, there was no relation at low tHcy levels, but a positive relation existed at medium and high tHcy concentrations. This indicates that the effects of high tCys on risk of coronary heart disease are dependent on the associated levels of tHcy. For cerebrovascular and peripheral vascular disease, low tCys was associated with increased risk at all tHcy levels, which indicates that low tCys levels are associated with cerebrovascular and peripheral vascular disease independently of tHcy.

Previous studies18–20,33 have demonstrated increased tCys levels in patients with myocardial infarction,18 cerebral infarction,19 or peripheral vascular disease.20 In addition, a recent study showed that plasma tCys is independently associated with cardiovascular diseases as well as with atherosclerotic lesions in hyperlipidemic patients.33 These studies, however, did not adjust for tHcy and other determinants of tCys.

Our results are partly in agreement with published studies on tCys and cardiovascular risk. We found a weak relation with coronary heart disease that was attenuated after adjustment for tHcy. In addition, a new finding of the present study is the association of low tCys levels with cerebrovascular and peripheral vascular disease, independent of tHcy level.

In conclusion, using data from a large European case-control study, we demonstrate that tCys is associated with vascular disease in the coronary, cerebral, and peripheral arteries. The relationship was strong and U-shaped for cerebrovascular and peripheral vascular disease, whereas for coronary heart disease, the relationship was weak and positive. Large prospective studies are needed to confirm our results and to elucidate the possible interaction between cysteine and homocysteine in the pathogenesis of cardiovascular disease.

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References


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