Hyperhomocysteinemia and Hypofibrinolysis in Young Adults With Ischemic Stroke

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Background and Purpose—Data from epidemiological and case-control studies suggest that increased total homocysteine (tHcy) levels are associated with increased risk for thromboembolic disease. The mechanisms by which hyperhomocysteinemia contributes to thrombogenesis are incompletely understood. The main objectives of this study of young ischemic stroke patients were (1) to examine fasting and post–methionine load levels of tHcy, (2) to ascertain the genotype frequency of the C677CT mutation in the methylenetetrahydrofolate reductase gene (TT genotype), and (3) to study the possible interaction between plasma tHcy levels and fibrinolytic factors.

Methods—This case-control study was based on 80 consecutive patients aged 18 to 44 years admitted between January 1992 and May 1996 as a result of a first-ever ischemic stroke. Forty-one healthy control subjects were recruited. Measurement of fasting tHcy and post–methionine load levels and evaluation of the fibrinolytic system were undertaken at least 3 months (mean, 5.1±1.9 months) after admission. Genotyping of the methylenetetrahydrofolate reductase gene was performed.

Results—Although the increase after methionine loading (ie, postload tHcy minus fasting-level tHcy) was significantly higher among patients, there was no difference in fasting and postload tHcy levels. After adjustment for conventional risk factors, elevated postload increase tHcy levels were associated with a 4.8-fold increased risk of ischemic stroke. There was no difference between patients and control subjects in either TT genotype frequency or T allele frequency. Abnormal response to methionine loading was associated with higher tissue plasminogen activator (tPA) mass concentration, higher plasminogen activator inhibitor-1 levels, and lower tPA activity. After adjustment for age, sex, body mass index, serum cholesterol, and triglycerides, an abnormal increase in postload tHcy levels remained significantly associated with tPA mass concentration levels (P=0.03).

Conclusions—A moderately elevated increase in tHcy levels after methionine loading was associated with an increased risk for ischemic stroke in young adults. In contrast, fasting tHcy levels did not differ between patients and controls. A moderately elevated increase in tHcy after methionine loading may provide additional thrombogenic risk mediated in part by interactions with the fibrinolytic system. In young stroke patients, a methionine loading test to detect hyperhomocysteinemia should always be considered in the convalescent phase of the disease. (Stroke. 1999;30:974-980.)

Key Words: homocysteine • fibrinolysis • mutation • young adults • stroke, ischemic

The importance of aberrant homocysteine metabolism for the development of cardiovascular disease, including ischemic stroke, has been suggested. However, very little is known of the association between total homocysteine (tHcy) levels and ischemic stroke in young adults, a subgroup of stroke patients in whom pathogenetic mechanisms are often uncertain or unknown. Genetic and nutritional factors are important determinants of homocysteine metabolism. These include milder impairment of enzymes involved in homocysteine metabolism as well as vitamin deficiencies, including folate, cobalamin and B6 deficiency. The common homozygotic mutation, C677T, in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene occurs in approximately 12% of most white populations. The mutation in the MTHFR gene has been proposed as a candidate risk factor for cerebrovascular disease, but this has not been verified in elderly stroke patients. Whether a mutation in the MTHFR gene is more common in young adults with ischemic stroke has not yet been studied.

The mechanisms by which homocysteine may increase the risk of vascular disease have not yet received an indisputable explanation. However, results from in vitro studies suggest that homocysteine may exert a procoagulant effect by an alteration of the activity of tissue plasminogen activator.
(tPA)\(^9\)\(^10\) and an impairment of von Willebrand factor (vWF) secretion.\(^11\) The in vitro nature of these studies underlines the need for more detailed investigations in vivo. We have recently shown that young stroke patients have low tissue plasminogen activity and high plasminogen activator inhibitor-1 (PAI-1) activity and tissue plasminogen mass concentration.\(^12\) It is therefore of interest to study possible associations between homocysteine levels and fibrinolytic factors.

The aims of this study of young adults with ischemic stroke were (1) to study possible abnormalities in total plasma homocysteine (tHcy), measured both in the fasting state and after methionine loading; (2) to assess whether the C677T mutation was associated with increased risk of ischemic stroke; and, finally, (3) to study possible associations between plasma tHcy levels and fibrinolytic factors.

Subjects and Methods

Northern Sweden is served by 13 local hospitals and a university hospital. The study population consisted of patients aged 18 to 44 years admitted to the University Hospital of Umeå between January 1991 and May 1996 as a result of first-ever ischemic stroke. From a consecutive series of 107 patients referred during this period,\(^3\) 80 patients (50 men, 30 women) enrolled from January 1992 to May 1996 participated in the present study. The method for analysis of plasma tHcy was not available for routine use during the first year of the study, which explains 19 of the 27 exclusions. Three exclusions were due to early death, 2 patients were not available for follow-up, and in 3 patients there was a lack of data. The inclusion/exclusion criteria and a detailed description of the diagnostic evaluation in the study, which explains 19 of the 27 exclusions. Three exclusions were due to early death, 2 patients were not available for follow-up, and in 3 patients there was a lack of data. The inclusion/exclusion criteria and a detailed description of the diagnostic evaluation in the study, which explains 19 of the 27 exclusions. Three exclusions were due to early death, 2 patients were not available for follow-up, and in 3 patients there was a lack of data.

Hypertension was defined as systolic blood pressure of $\geq 160$ mm Hg and/or diastolic pressure of $\geq 95$ mm Hg on 2 separate occasions measured out of the acute phase of stroke or treatment with antihypertensive drugs for $>2$ weeks before recruitment; the diagnosis of diabetes mellitus was established by medical records or at recruitment according to WHO criteria.\(^14\) Current smoking was defined as smoking at least 1 cigarette a day for at least 2 months.

At the time of sampling, 9 patients were on treatment with oral anticoagulants (coumarin derivatives). Seventy-four patients received a low dose of aspirin as antiplatelet medication. Two patients were taking B-vitamin supplementation at the time of follow-up. Blood sampling, including samples for homocysteine and fibrinolytic studies, was undertaken at a follow-up visit at least 3 months (mean, 5.1 ± 1.9 months) after admission.

Control subjects (n = 41) were recruited by local announcements through the university hospital faculty and staff members and the Umeå community at large after excluding those individuals receiving medical treatment or giving a history of diseases associated with increased risk for cerebrovascular disease. All controls were free of overt disease according to a questionnaire, including no regular medical intake and no known history of cerebrovascular disease.

Blood Sampling and Laboratory Methods

Sampling took place in the early morning (before 9 AM) after overnight fasting. Coffee drinking or smoking was not allowed on the morning of sampling. Venous blood samples obtained from the groups were drawn from the antecubital vein without stasis, after 10 minutes of bed rest, into evacuated glass tubes (Vacutainer) containing 1/100 volume of 0.5 mol/L EDTA for the homocysteine measurement in the fasting state and for the fibrinolytic assays.\(^13\) into 1/10 volume of 0.45 mol/L citrate, pH 4.4 (Stabilyte tubes, Biopool).

After withdrawal of blood in the fasting state, patients and control subjects received a single dose of 0.1 g/kg L-methionine per os together with a standardized low-methionine breakfast. A second blood sample was taken 4 hours after the methionine load. We refer to the difference between these 2 concentrations as the increase in tHcy. During the 4-hour period, the patients were only allowed to ingest food poor in protein and methionine.

Plasma and serum aliquots were prepared by centrifugation at 1500g for 15 minutes at room temperature and stored within 1 hour at $-80^\circ C$ until assayed.

Biochemical Analyses

Plasma tHcy concentrations were determined in EDTA-plasma by high-pressure liquid chromatography using electrochemical detection. Dithiothreitol was used to reduce disulphide bonds, yielding free homocysteine. The samples were then deproteinized with trichloroacetic acid. In the concentration range of 10 to 50 $\mu$mol/L, the interassay coefficients of variation was 3.6%.

Plasma levels of each hemostatic factor were determined using the following assay systems: the mass concentration of tPA in plasma was determined with enzyme-linked immunosorbent assay.\(^16\) The reagent kit (Imulys tPA) was purchased from Biopool. The activities of tPA and PAI-1 were measured with chromogenic substrate assay based on the fibrin-stimulated, tPA-mediated plasminogen-to-plasmin conversion.\(^17\) The reagent (Spectrolyse fibrin) was purchased from Biopool. vWF was measured with an enzyme-linked immunosorbent assay,\(^18\) utilizing reagents purchased from DAKO; the values are expressed as a percentage of the value obtained in a pool of normal subjects. Plasma fibrinogen was measured with a thrombin reaction time kit from BioMérieux.

Serum total cholesterol, HDL cholesterol, and triglycerides were analyzed on a multianalyzer (Vitros 950 IRC, Johnson & Johnson, Clinical Diagnostics Inc). LDL cholesterol levels were calculated using the Friedewald formula. Lipoprotein(a) was analyzed with an enzyme-linked immunosorbent assay using polyclonal antibodies to Lp(a) homos in goat. The detection level of Lp(a) was 10 mg/L.

Among white patients, a Lp(a) lipoprotein level of $\geq 300$ mg/L has been established as a risk threshold for the development of cardiovascular disease.\(^19\) Serum folate and cobalamin levels were analyzed with DPC Daulcount or Quantaphase II B12/folate radioassay (BioRad, Diagnostics Group). The correlation between the methods was good. In the patient group serum folate and cobalamin levels was measured at the time of diagnosis as well as in the convalescent phase. Vitamin B\(_6\) (pyridoxal 5'-phosphate; PLP) was measured by enzymatic photometry with high-pressure liquid chromatography separation\(^20\) by MIMELAB-AB.

Genotyping

DNA was extracted according to Caddy et al\(^2\) after cell lysis, deproteinization with perchlorate, and extraction with chloroform and resin, using the Nucleon DNA extraction kit from Nucleon Biosciences. The extracted DNA was stored at $-80^\circ C$ until analysis. The DNA samples were subjected to amplification by the polymerase chain reaction and the restriction enzyme Hinf I was used to identify those with the C677T mutation, as described by Frooss.\(^22\) The mutant allele was designated as “T” and the wild-type as “C”.

Statistical Methods

Means and proportions were computed for background variables. Comparisons between patients and controls were made with the Student t test or Mann-Whitney test for continuous variables. The $\chi^2$
or Fisher exact test was used to test differences in proportions. Spearman correlation coefficients were used to measure correlations between continuous variables. Kruskal-Wallis 1-way ANOVA was used for comparisons of continuous variables between diagnostic subgroups.

Geometric means, medians, and interquartile range (25th and 75th percentiles) were computed for total LDL and HDL cholesterol, triglycerides, Lp(a), serum folate, cobalamin, PLP, and tHcy levels due to skewed distribution of the variables. For multivariate analysis, ANOVA with covariates was used with logarithmically transformed dependent variables. From the ANOVA models, adjusted geometric means were computed.

Odds ratios with 95% confidence intervals for ischemic stroke were calculated for categorized fasting, postload, and postload increase tHcy levels. The cutoff point was set at the 90th percentile of the homocysteine distribution in the healthy control subjects. Adjusted ORs were calculated with a logistic regression model with selected independent variables. Two-tailed tests were used, and a value of \( P < 0.05 \) was considered significant.

Informed verbal consent was obtained from all subjects. The study was approved by the Research Ethics Committee of Umeå University, and the data handling procedures were approved by the National Computer Data Inspection Board.

**Results**

**Etiology of Stroke**

The patient population comprised 50 men and 30 women. Atherosclerotic vasculopathy was diagnosed as the cause of cerebral infarction in 10 patients. In 6 patients only discrete plaque formation in the carotid arteries, without any signs of flow abnormalities, was demonstrated. In 4 patients an atherosclerotic stenosis of >50% was found. In addition, transesophageal echocardiography revealed a simple aortic arch atheroma in 3 patients. Arterial dissection was diagnosed in 18 patients, affecting the internal carotid artery in 9 patients and the vertebral artery in 9. A cardioembolic etiology was presumed in 29 patients. The most frequent abnormality was right-to-left cardiac shunts consistent with patent foramen ovale (PFO), found in 25 patients. Atrial septum aneurysm, detected in 5 patients, was isolated in 3 patients and associated with PFO in 2. With respect to hematologic causes of stroke, 1 patient had an inherited protein S deficiency and low anticardiolipin IgG titer. In 1 patient the ischemic stroke occurred in the postpartum state. Four patients met the criteria for lacunar infarct. Oral contraceptive use was the likely cause of stroke in 3 female patients. The etiology of cerebral infarction was indeterminate in 13 patients.

**Homocysteine Levels**

Table 1 summarizes the basic clinical features, homocysteine levels, and other risk factors among patients and control subjects. There was no difference in fasting and postload tHcy levels between patients and controls. However, the increase after methionine loading (ie, postload minus fasting tHcy level) was significantly higher among patients. Table 2 shows the number of patients and controls with elevated tHcy levels. Of the 30 patients with elevated postload increase of tHcy, 7 were also defined as having elevated fasting tHcy. Four patients had isolated fasting homocysteinemia. Thus, a total of 34 patients (42%) fulfilled the criteria for hyperhomocysteinemia. The Figure shows that for postload tHcy peak levels, the distribution among patients was shifted toward the right across the full range of values compared with that among controls. Fasting tHcy and postload tHcy levels were analyzed according to the 4 main diagnostic categories for analysis of a possible diagnostic dependency. The other diagnostic categories were excluded because of small numbers. The diagnostic subgroups did not differ significantly from each other with respect to either fasting or postload levels of tHcy (data not shown).

**Associations With Cerebrovascular Risk Factors**

Hypertension and diabetes mellitus were present in 28% and 4%, respectively, of the patients. On univariate analysis, patients had higher circulating fibrinogen, total and LDL cholesterol, and triglycerides, and PLP levels significantly differed between patients and control subjects. There was no difference in mean levels of Lp(a) between patients and controls. Seventeen (21.5%) of the patients had an Lp(a) lipoprotein of \( \geq 300 \) mg/L, as did 7 (17.1%) of the controls (\( P = 0.7 \); OR = 1.3; 95% CI, 0.5 to 3.5). There were no significant differences between patients and controls regarding vWF, serum folate, and cobalamin levels.

**TABLE 1. Demographic Characteristics, Vascular Risk Factors, and Laboratory Findings**

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>80</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>36.2±6.2</td>
<td>35.5±6.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Gender, % male</td>
<td>62</td>
<td>49</td>
<td>0.2</td>
</tr>
<tr>
<td>Body mass index (kg/m2)</td>
<td>25.7±3.7</td>
<td>24.4±4.1</td>
<td>0.08</td>
</tr>
<tr>
<td>n(%) current smokers</td>
<td>24</td>
<td>22</td>
<td>0.8</td>
</tr>
<tr>
<td>OCU, % female</td>
<td>26.6</td>
<td>28.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>3.08±0.79</td>
<td>2.31±0.53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>vWF, %</td>
<td>109.6±34.8</td>
<td>116.3±37.8</td>
<td>0.35</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.82±0.97</td>
<td>4.86±0.83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.68±0.87</td>
<td>1.28±0.74</td>
<td>0.003</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.31±0.31</td>
<td>1.22±0.34</td>
<td>0.15</td>
</tr>
<tr>
<td>LDL, mmol/L</td>
<td>3.79±0.89</td>
<td>3.04±0.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lp(a), mg/L</td>
<td>164.3±202.7</td>
<td>160.2±177.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Serum folate, mmol/L</td>
<td>12.0±7.5</td>
<td>9.61±4.4</td>
<td>0.06</td>
</tr>
<tr>
<td>Serum cobalamin, pmol/L</td>
<td>321.5±106.6</td>
<td>352.9±132.4</td>
<td>0.2</td>
</tr>
<tr>
<td>PLP, nmol/L</td>
<td>19.6±7.3</td>
<td>16.7±6.3</td>
<td>0.02</td>
</tr>
<tr>
<td>Fasting tHcy, μmol/L</td>
<td>12.88±6.06</td>
<td>13.23±7.49</td>
<td>0.46</td>
</tr>
<tr>
<td>Postload tHcy, μmol/L</td>
<td>25.42</td>
<td>32.55</td>
<td>0.10</td>
</tr>
<tr>
<td>Postload increase in tHcy, μmol/L</td>
<td>22.9</td>
<td>19.64</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Mean±SD values and geometric means (italics) are shown. OCU indicates oral contraceptive use.

* t test for continuous variables; \( \chi^2 \) or Fisher exact test for proportions.
Univariate correlation coefficients between risk factors and tHcy levels are shown in Table 3. BMI and triglyceride levels were significantly correlated with both fasting and postload tHcy increase, whereas total cholesterol, LDL cholesterol, and fibrinogen levels were significantly correlated only with postload tHcy increase. Fasting tHcy levels were higher among smokers ($P=0.004$; $P=0.02$), whereas the postload tHcy increase did not differ between smokers and nonsmokers.

**Plasma tHcy and Risk of Ischemic Stroke**

The ORs of ischemic stroke for subjects with elevated tHcy relative to subjects with levels at or below the cutoff points are given in Table 2. Elevated post load tHcy increase levels were associated with a 5.9-fold increased risk of ischemic stroke after adjustment for age and gender. After adjustment for possible confounding factors, OR for elevated postload tHcy increase levels remained significant, with a 4.8-fold increased risk of ischemic stroke.

**Vitamin Levels and tHcy**

Levels of all vitamins correlated inversely with plasma fasting and postload tHcy in a similar way for patients and controls. Across the whole population, fasting tHcy was significantly inversely correlated with serum folate ($r_s=-0.28$, $P=0.002$) but not with cobalamin levels ($r_s=-0.15$, $P=0.09$). Plasma PLP was inversely but not significantly correlated with postload tHcy increase ($r_s=-0.18$, $P=0.06$). There was no difference between serum folate and cobalamin measured at time of diagnosis and follow-up (data not shown).

**MTHFR Genotype**

In the total study population, the allele frequency of the mutation was 25%. There was no difference between patients and control subjects in either (TT) genotype frequency (13.5% versus 7.5%; OR=1.43; 95% CI, 0.4 to 5.5) or (T) allele frequency (26% versus 24%; OR=0.97; 95% CI, 0.5 to 1.9). Fasting or postload tHcy levels did not differ between the genotypes (data not shown).

**Homocysteine and Fibrinolysis**

Tissue plasminogen activity was lower whereas plasminogen levels, tPA mass concentration, and PAI-1 mass concentration were higher in the patient group (data not shown). Univariate correlation coefficients ($r_s$) for baseline fibrinolytic variables versus postload tHcy increase in patients ($n=80$), controls ($n=41$), and across the whole study group ($n=121$) are shown in Table 4. There were highly significantly correlations between postload tHcy increase and the fibrinolytic variables across the whole group.

In the entire study population, an abnormal increase in postload tHcy levels (>90th percentile) was associated with higher plasminogen, tPA mass concentration, and PAI-1
levels and lower tPA activity (Table 5). After adjustment for age, sex, BMI, serum cholesterol, and triglycerides, an abnormal increase in postload tHcy levels remained significantly associated with plasminogen (P = 0.001) and tPA mass concentration levels (P = 0.03). There was no significant increase in vWF levels among subjects with an abnormal postload tHcy increase.

**Discussion**

The main finding in this study was that the increase in postload tHcy levels was independently associated with ischemic stroke after adjustment for established risk factors. In contrast, fasting tHcy levels did not differ between patients and controls. Increase of postload homocysteine levels above the 90th percentile of the control distribution were observed in 37.5% of the patients.

There have been 3 prospective studies of the relationship between fasting tHcy and risk of stroke in middle-aged populations.23–25 The evidence derived from these studies has been contradictory. Two studies thus failed to demonstrate an increased risk of cerebrovascular disease with higher fasting tHcy levels.23,24

A few case-control studies26–30 have provided more detailed information with respect to homocysteine metabolism in younger stroke patients (<60 years), whereas no previous study has examined young adults aged <45 years with ischemic stroke (Table 6). Fasting levels for homocysteine have been reported in only 1 study27 with a limited number of patients aged <55 years with cerebrovascular disease. In this study, fasting homocysteine levels were not significantly higher than controls in the subgroup defined as cerebral thrombosis as opposed to patients with cerebrovascular disease on the basis of carotid artery disease. Clearly, different subsettings have been evaluated in earlier studies, and a selection bias due to different referral patterns and inclusion criteria is possible. Furthermore, either the studies have been performed at different time points in the poststroke phase or the time of sampling has not been stated (Table 6).

Most recently, a prospective study,31 restricted to subjects with systemic lupus erythematosus, reported on the association between fasting homocysteine and risk of stroke in a comparable age group. After adjustment for established risk factors, hyperhomocysteinemia remained an independent risk factor for stroke.

Determination of only fasting tHcy will fail to identify the substantial proportion of subjects who have normal fasting but elevated postload tHcy levels.30,32,33 In our study a large proportion (23/30 = 76%) of patients with abnormal homocysteine metabolism would have remained undetected by measuring fasting tHcy alone. These results emphasize that a normal fasting tHcy concentration is not synonymous with normal homocysteine metabolism in a young stroke population and that methionine loading is required for the diagnosis of hyperhomocysteinemia. The reason for the high frequency of abnormal postmethionine loading tHcy is unclear. Vitamin B6 deficiency may contribute to an abnormal methionine loading test.34 However, the increase of postload homocysteine levels in our patients was not explained by a subnormal PLP status. In fact, PLP levels were higher among patients. It is known that individuals who are homozygous for the C677T mutation in MTHFR need higher vitamin cofactor concentrations for normal function.35 A genotype-dependent functional deficiency in patients with “normal” vitamin levels due to polymorphisms in the transsulfuration pathway might be one explanation. Vitamin treatment might be of value in individuals in whom functional vitamin deficiency is suspected. The treatment can be evaluated and optimized by repeating the methionine loading test.

A limitation of all case-control studies is that one cannot rule out the possibility that elevated levels of tHcy may be influenced by the disease, underlying vascular disease, or its treatment. Recent data suggest that an acute stroke36 and acute myocardial infarction37 alter levels of tHcy, which could affect the apparent association with risk of disease. Plasma tHcy levels were lower in the acute phase than in the convalescent phase after stroke and myocardial infarction, measured at a median interval of 1.5 years after the stroke and

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**TABLE 3.** Spearman Correlation Coefficients for Cerebrovascular Risk Factors Versus Fasting tHcy and tHcy Increase After Methionine Loading

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Fasting tHcy</th>
<th>Postload tHcy Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index</td>
<td>0.20*</td>
<td>0.32†</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.23*</td>
<td>0.35†</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.14</td>
<td>0.24‡</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.10</td>
<td>0.23*</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.05</td>
<td>0.18*</td>
</tr>
</tbody>
</table>

*P<0.05; †P<0.001; ‡P<0.01.

**TABLE 4.** Postload tHcy Increase: Spearman Correlation Coefficients in 80 Patients and 41 Controls and Across the Whole Group

<table>
<thead>
<tr>
<th></th>
<th>Postload tHcy Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients (n=80)</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>0.19</td>
</tr>
<tr>
<td>tPA activity</td>
<td>-0.20</td>
</tr>
<tr>
<td>tPA mass concentration</td>
<td>0.28†</td>
</tr>
<tr>
<td>PAI-I</td>
<td>0.22‡</td>
</tr>
</tbody>
</table>

*P<0.001; †P<0.01; ‡P<0.05.

**TABLE 5.** Fibrinolytic Variables Among Subjects With Normal Increase in tHcy After Methionine Load (<90th Percentile of Control Group) and in Subjects With Postload ≥90th Percentile

<table>
<thead>
<tr>
<th></th>
<th>Increase After Load &lt;90th Percentile</th>
<th>Increase After Load ≥90th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median Range</td>
<td>Median Range</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>1.7</td>
<td>1.5–1.8</td>
</tr>
<tr>
<td>tPA activity</td>
<td>0.1</td>
<td>0.1–0.2</td>
</tr>
<tr>
<td>tPA mass concentration</td>
<td>6.8</td>
<td>4.6–10.9</td>
</tr>
<tr>
<td>PAI-I</td>
<td>8.6</td>
<td>4–15.8</td>
</tr>
</tbody>
</table>

Range indicates interquartile range (25th and 75th percentiles).

*Mann-Whitney U test.
6 weeks after the acute myocardial infarction, respectively. Thus, it seems unlikely that this phenomenon can explain our findings of similar fasting tHcy levels in patients and controls, because we measured tHcy in the convalescent phase. The behavior of postload levels in the acute phase and in the convalescent phase after stroke has not been studied.

Medication prescribed for patients with stroke may also modify tHcy levels. It has been suggested that the use of acetylsalicylic acid or other antithrombotic drugs may decrease tHcy levels. However, in patients with coronary heart disease, tHcy changes during follow-up were not related to antithrombotic treatment. Dietary change is another potential source of bias, but neither serum folate nor cobalamin level in our patient group changed during the follow-up period.

The postload tHcy increment was associated with lower tPA activity, higher PAI-1 activity, and independent of conventional risk factors, higher tPA mass concentrations. This may indicate that an interaction with the fibrinolytic system may be one mechanism by which tHcy can provoke thromboembolic events. This is in agreement with an earlier study showing a similar relationship between increased post–methionine load tHcy levels and a significantly increased euglobulin clot lysis time, ie, corresponding to low tPA activity and increased PAI-1 activity. Whether these effects are present as a direct effect on endothelial cells is not clear. We did not find vWF, a marker for endothelial dysfunction, to be associated with postload tHcy increase above the 90th percentile. This suggests that high plasma levels of homocysteine do not measurably influence the endothelium or that this marker is not sensitive enough to detect the very early phase of homocysteine-induced endothelial injury. Impaired endothelium-dependent vasodilatation associated with elevated tHcy has been demonstrated in vivo. In healthy human volunteers, an acute increase in plasma homocysteine after a methionine challenge has been found to be associated with substantial impairment of endothelium-dependent, flow-mediated dilatation in an inverse and linear manner. The resultant endothelial dysfunction may then eventually contribute to vasospasm and thrombosis.

tHcy levels in the various etiological subgroups did not differ significantly. This indicates that abnormal homocysteine metabolism in premature ischemic stroke is not associated with a particular etiology, eg, atherosclerosis. However, abnormal homocysteine metabolism under varying circumstances may provide an additional thrombogenic risk, possibly in part mediated by interactions with the fibrinolytic system.

The association of genetic abnormalities in homocysteine metabolism and risk of stroke is inconclusive at present. We found no association between ischemic stroke and TT genotype, but it is difficult to draw firm conclusions in this respect, taking into account the relatively small number of study subjects.

Lipoprotein(a) has been advocated as a marker of prothrombotic risk in cardiovascular disease. Our data do not suggest that Lp(a) contributes to the risk of stroke in young adults. This agrees with a recent prospective study in which no association was found between plasma concentration of Lp(a) and future risk of stroke.

In conclusion, our data suggest that an exaggerated tHcy increase after methionine loading represents a cerebrovascular risk factor in premature ischemic stroke. This association was present also after adjustment for other conventional cerebrovascular risk factors, including fibrinogen. Our data furthermore suggest that homocysteinemia after methionine loading is associated with a hypofibrinolytic state. Homocysteine may thus participate as an additional “hit” in abnormalities in coagulation and vascular cell functions in young adults with ischemic stroke.

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