Elevated Plasma Homocyst(e)ine Concentration as a Possible Independent Risk Factor for Stroke

Bruce M. Coull, MD, M. Rene Malinow, MD, Nancy Beamer, MS, Gary Sexton, PhD, Frank Nordt, PhD, and Pat de Garmo, ANP

Homocyst(e)ine refers to the sulfur-containing amino acids homocysteine, homocystine, and homocysteine-cysteine mixed disulfide, which normally exist in plasma in both the free and protein-bound forms. Marked hyperhomocyst(e)inemia is associated with well-recognized complications of occlusive thrombotic events and a characteristic syndrome. It is less clear whether mild to moderate elevations in plasma homocyst(e)ine concentrations (i.e., 1.5–5-fold increases) also represent a risk factor for stroke and, if so, whether it is independent of other recognized risk factors. To examine these questions we compared the plasma homocyst(e)ine levels in 41 patients with acute strokes, 27 patients with transient ischemic attacks, 31 patients with recognized risk factors for but no recent symptoms of cerebrovascular disease, and 31 normal volunteers (controls). Plasma homocyst(e)ine concentration was moderately but significantly higher in the patients than in the controls ($p<0.0001$). Approximately 30% of the patients had homocyst(e)ine levels higher than the controls. No relation was found between homocyst(e)ine concentration and other recognized stroke risk factors or stroke type; however, a positive correlation was found between serum uric acid and plasma homocyst(e)ine levels. These data suggest that a moderately elevated plasma homocyst(e)ine concentration may be an independent risk factor for cerebrovascular disease. (Stroke 1990;21:572–576)

Homocysteine, a thiol-containing amino acid derived from the metabolism of methionine, is readily oxidized in plasma to the disulfide homocystine and to homocysteine-cysteine mixed disulfide. All three chemicals occur normally in plasma in both the free and protein-bound forms and are collectively referred to as homocyst(e)ine. Brain infarction, carotid artery occlusion, and premature arteriosclerosis are well-recognized complications of marked hyperhomocyst(e)inemia caused by several inborn errors of metabolism including a deficiency of cystathionine $\beta$-synthase. The characteristic clinical syndrome that accompanies this autosomal recessive trait manifests early in life, and most cases are recognized by adolescence. In this form of the disease, plasma levels of homocyst(e)ine are markedly elevated (usually >20 times normal) and there is associated homocystinuria. In contrast, mild to moderate elevations in homocyst(e)ine concentrations (approximately 1.5–5 times normal) are recognized in persons with partial reductions in cystathionine $\beta$-synthase activity and those with nutritional deficiencies that influence the metabolism of methionine and homocysteine. Recent studies suggest that these individuals may be at increased risk for developing the complications of arteriosclerotic cardiovascular and peripheral vascular disease, including stroke. Among 36 subjects (19 with atherosclerotic cerebrovascular disease [CVD]), Brattstrom et al found moderate elevations in plasma mixed disulfide concentrations in a significant proportion of cases with CVD. Although the authors argue that this elevation reflects a metabolic abnormality, they did not examine its relation with other recognized stroke risk factors or stroke type. We compared the risk factors for stroke and measured the homocyst(e)ine levels in patients with acute brain infarction (stroke), transient ischemic attacks of the brain (TIAs), or recognized stroke risk factors but no recent cerebrovascular symptoms with those in individuals of similar age and sex without recognized stroke risk factors or CVD.

From the Department of Neurology, Veterans Administration Medical Center (B.M.C.), the Department of Neurology, Oregon Health Sciences University (B.M.C., N.B., G.S., F.N., and P.d.G.), Portland, and the Oregon Regional Primate Research Center (M.R.M.), Beaverton, Oregon.

Supported in part by National Institutes of Health grant NS17493 and The Fred Meyer Charitable Trust.

Address for correspondence: Bruce M. Coull, MD, Neurology Service (127-P), Veterans Administration Medical Center, PO Box 1034, Portland, OR 97207.

Received July 6, 1989; accepted December 4, 1989.
Subjects and Methods

We recruited 130 subjects into four groups. Patients with acute stroke (n=41), those with TIAs (n=27), and those at risk (see below) for CVD (n=31) were enrolled through the Oregon Health Sciences University and Portland Veterans Administration Medical Center Hospital and Clinics.

Acute stroke patients were eligible for the study if they had experienced a brain infarction without evidence of intracerebral hemorrhage on computed tomography (CT) or magnetic resonance imaging (MRI), venous blood was obtained ≤72 hours after ictus. Patients with TIAs were entered if they had experienced an attack (including transient monocular blindness) within the previous 2 weeks. Patients were entered into the at risk group if they had a history of and/or received treatment for hypertension, diabetes mellitus, cardiac diseases known to be associated with cerebral embolic events, tobacco use, or cerebrovascular atherosclerosis (identified by the presence of carotid stenosis on either Doppler ultrasonography or cerebral angiography). The at risk group included persons with previous stroke or TIA who had been without cerebrovascular symptoms for the 6 months prior to entry. Most (24 of 31, 77%) at risk patients had two or more stroke risk factors.

A control group of 31 normal volunteers was recruited through the Oregon Health Sciences University faculty and staff and the Portland community at large after excluding those receiving medical treatment or giving a history of diseases associated with increased risk for cerebrovascular events. Volunteers were admitted to the control group only if a screening medical questionnaire, a complete blood count and differential, and laboratory studies (including electrolytes, blood urea nitrogen (BUN) and creatinine, cholesterol, bilirubin, and liver enzymes) indicated no abnormality. Homocyst(e)ine levels in the control group have been reported.

Informed consent was obtained from all 130 subjects according to the policies of the Institutional Review Board of the Oregon Health Sciences University and the Portland Veterans Administration Medical Center. Approximately 300 items of information describing each subject were accessed through the Oregon Stroke Center Assessment Registry. This data repository includes information on stroke risk factors, clinical presentation and diagnosis, hematologic and blood chemistry values, results of carotid ultrasonography, brain CT, MRI, echocardiography, and cerebral angiography examinations if performed, family medical history, and selected demographic data.

For the stroke and TIA groups, all clinical, laboratory, and radiographic data was reviewed to classify the most probable explanation of symptoms as thromboembolic (large-vessel atheroembolism), cardioembolic (the heart as the most probable source of thromboemboli), or lacunar (arteriolar occlusive disease of the brain).

Homocyst(e)ine concentration was determined using a modification of the method of Smolin and Schneider as described previously. In brief, highly sensitive high-performance liquid chromatography was performed with a BAS 400 LEC chromatograph (BioAnalytical System, West Lafayette, Indiana) equipped with a single-piston pump and a single gold/mercury electrode at a potential of 0.15 V. A 200-μl aliquot of plasma was diluted with 100 μl of water and mixed with 300 μl of 9 M urea (pH 9.0), 50 μl of n-amyl alcohol, and 50 μl of 10% sodium tetraborohydrate in 0.1 M NaOH. After 30 minutes at 50° C, the proteins were precipitated with 600 μl of 20% trichloroacetic acid.

Standards comprised 100 μl of various L-homocysteine solutions added to pooled plasma, replacing the 100 μl of water; the concentrations of added homocysteine (Fluka Chemical Corp, Ronkonkoma, New York) were defined after the addition of all diluents required for reduction and deproteinization. Standard curves were run in duplicate at the beginning, middle, and end of each daily run. Homocyst(e)ine concentration was calculated from the height of peaks in the standard curves. The results are expressed as nanomoles homocysteine per milliliter (i.e., 2×homocystine concentration).

The mean and standard deviation were calculated for each variable by group. Because the distribution of homocyst(e)ine levels was found to be skewed to the right, the natural logarithm transformation was used to improve normality. Transformed as well as untransformed data were analyzed in parallel. The groups were compared using one-way analysis of variance followed by Bonferroni post hoc procedures. Because there were no qualitative differences in the results, untransformed homocyst(e)ine values are reported with their significance levels. Moreover, since women are reported to have lower homocyst(e)ine levels than men, the data were also analyzed for gender differences. Correlations between homocyst(e)ine levels and stroke risk factors were quantified using regression analysis.

Results

Table 1 shows the mean±SD homocyst(e)ine level and demographic and risk factor data for each group. Controls were slightly but not significantly younger than patients in the other three groups. The lowest cholesterol levels were found in the stroke group, whereas the three patient groups had significantly higher mean levels of glucose, BUN, and uric acid than the control group. Transformed homocyst(e)ine levels differed between the stroke (p<0.0001), TIA (p<0.007), and at risk groups (p<0.0001) compared with the controls. The untransformed homocyst(e)ine data for individual subjects and the mean values for each group are shown in Figure 1.

With respect to specific stroke risk factors, no relation was found between homocyst(e)ine level and hypertension, diabetes mellitus, degree of carotid stenosis, cigarette smoking, alcohol consumption, or...
intermittent claudication. As shown in Figure 2, a significant direct correlation was found between the serum levels of uric acid and the plasma levels of homocyst(e)ine.

Of the 68 patients with stroke or TIA, 40 were classified as having thromboembolic, 11 as having cardioembolic, and 12 as having lacunar mechanisms for their ischemia; in the remaining five patients the clinical data were either inconclusive or insufficient for accurate classification of the mechanism. Of the patients with defined mechanisms, homocyst(e)ine levels were slightly higher in the lacunar subgroup, but in this small sample the differences were not significant.

Analysis of the data excluding the women did not change the results appreciably. No sex differences were found for any variable. The homocyst(e)ine levels for men in the four groups were 16.6±5.8, 15.6±6.1, 15.5±6.4, and 11.1±3.3 nmol/l for the stroke, TIA, at risk, and control groups, respectively. Differences in transformed homocyst(e)ine levels between men with stroke \( \textit{p}<0.0009 \), those with TIA \( \textit{p}<0.05 \), and those at risk for CVD \( \textit{p}<0.02 \) compared with male controls remained significant.

**Discussion**

Our study suggests that significantly elevated total homocyst(e)ine concentration may exist in up to a third of patients with stroke or TIA. In a previous study, moderate hyperhomocyst(e)inemia was found in a similar proportion of patients with symptomatic peripheral vascular disease; those patients also had a high incidence of symptomatic carotid atherosclerosis.

Since our study is cross-sectional, we do not know if homocyst(e)ine levels remain elevated. However, several implications can be drawn from our observation that homocyst(e)ine concentrations do not differ among patients with stroke, those with TIA, and those at risk for CVD. The results in the at risk group suggest that an elevated homocyst(e)ine concentration is not an acute-phase reaction to a cerebrovascular event. The underlying cause for this elevation remains to be clarified, but it could reflect genetic or acquired deficiencies in the metabolic pathways for homocysteine metabolism. The observation that heterozygosity for homocystinuria exists in roughly 30% of young subjects with premature occlusive CVD buttresses the genetic hypothesis. That we were unable to detect a relation between a high homocyst(e)ine concentration and either high mean erythrocyte volume (which occurs in folate or vitamin B₁₂ deficiency) or low serum albumin levels suggests that nutritional deficiency is not the principal explanation for elevated plasma homocyst(e)ine levels.

We did not detect a significant sex difference in homocyst(e)ine levels in the control group. This probably reflects the fact that our female controls were postmenopausal (mean±SD age 57.3±10.6 years). Although young healthy women have homocyst(e)ine levels lower than healthy men, the difference diminishes with aging. Boers et al have suggested that highly efficient methionine metabolism in premenopausal women protects them against...
homocyst(e)ine accumulation. Studies by Kang et al, which show an “abrupt increase” in total plasma homocyst(e)ine in women after age 50 years, are consistent with this view. Thus, the sex difference in homocyst(e)ine levels largely disappears with aging and/or the development of atherosclerosis. Collectively, these observations suggest hormonal influences on plasma homocyst(e)ine regulation and/or metabolism in women.

Our finding of a direct relation between serum uric acid and plasma homocyst(e)ine levels is in agreement with the observation by Kang et al in subjects with coronary artery disease. Those investigators attributed this relation to coexisting renal failure and the use of diuretics. In our subjects, plasma homocyst(e)ine levels correlated with serum levels of uric acid (r=0.41, p<0.0001), BUN (r=0.37, p<0.0001), and creatinine (r=0.38, p<0.0001) but not with the use of diuretics or with hypertension. It is possible that elevated uric acid and homocyst(e)ine concentrations reflect renovascular atherosclerosis and the complications of systemic vascular disease. For example, in data from the Framingham Study Abbott et al found a significant relation between the presence of gout not related to diuretic use and coronary heart disease in men. In the same population, hyperuricemia was related to the occurrence of myocardial infarction. In support are data from Woliscroft et al, which show that hyperuricemia is associated with poor prognosis during acute illness and may reflect tissue hypoxia. Accordingly, it may be useful to investigate the relation between hyperhomocyst(e)inemia, elevated uric acid concentrations, and the long-term prognosis for cerebrovascular events.

To date, investigations have failed to explain how moderate hyperhomocyst(e)inemia causes vascular injury or pathologic thrombosis. Several lines of evidence suggest that the production of atheromaous lesions is instigated by homocyst(e)ine-induced injury to the endothelial surface. In animals, intravenous infusions of homocyst(e)ine damage vascular endothelium and stimulate the consumption of platelets. Studies indicate that the generation of hydrogen peroxide or the production of oxygen free radicals causes the endothelial injury. Furthermore, Starkebaum and Harlan have shown that in a copper-catalyzed reaction, hydrogen peroxide generated by homocyst(e)ine produces endothelial injury. Pivotal to this reaction is the affinity of the thiol group in homocysteine for copper, which leads to auto-oxidation. Although elevated plasma copper concentrations have been reported in persons with homocystinemia, normal plasma levels of ceruloplasmin are capable of promoting this reaction.

The automated methods used in this study are simple, inexpensive, and sufficiently sensitive to reliably identify individuals with moderate hyperhomocyst(e)inemia. Thus, this method can be applied as a screening tool in large populations at risk for CVD. The identification of these subjects has therapeutic implications for several reasons. Based on our results, moderate hyperhomocyst(e)inemia is an independent risk factor for stroke and TIA, and evidence from the literature indicates that even in subjects with normal serum folic acid levels the addition of supplementary folic acid to the diet can reduce moderately elevated homocyst(e)ine levels to normal, presumably by enhanced remethylation of homocyst(e)ine to methionine. Thus, inexpensive means are at hand to detect and to treat subjects with CVD who have this potentially independent risk factor for stroke. The benefits of this therapeutic approach and the long-term mechanistic understanding of how moderately elevated homocyst(e)ine concentrations contribute to stroke risk await additional longitudinal trials.

Acknowledgment

We thank Lynn Riley for her assistance in preparation of this manuscript.
References


KEY WORDS • cerebral infarction • homocysteine • risk factors
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doi: 10.1161/01.STR.21.4.572

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