A prospective study of plasma homocyst(e)ine and risk of ischemic stroke

A Prospective Study of Plasma Homocyst(e)ine and Risk of Ischemic Stroke

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Background and Purpose Several studies have reported elevated circulating homocyst(e)ine levels in subjects with cerebral atherosclerosis. We assessed prospectively whether high plasma levels of homocyst(e)ine affect risk of ischemic stroke and evaluated whether high blood pressure modifies any such effect.

Methods The study sample was drawn from the Physicians' Health Study, a randomized, double-blind, placebo-controlled trial of aspirin and beta-carotene in 22 071 US male physicians. A total of 14 916 subjects 40 to 84 years old with no prior history of stroke, transient ischemic attack, or myocardial infarction provided blood samples at baseline and were followed for 5 years, with 99.7% morbidity and 100% mortality follow-up. Using a nested case-control design, we assayed homocyst(e)ine in samples from 109 subjects who subsequently developed ischemic stroke and 427 control subjects.

Results The mean plasma concentration of homocyst(e)ine was slightly higher in subjects with stroke (11.1±4.0 [±SD] nmol/mL) than in control subjects (10.6±3.4 nmol/mL), but the difference was not statistically significant (P=.12). The crude odds ratio of ischemic stroke for subjects in the upper 20% (>12.7 nmol/mL) compared with those in the bottom 80% of homocyst(e)ine levels was 1.4 (95% confidence interval, 0.8 to 2.2). The odds ratio was 1.2 (95% confidence interval, 0.7 to 2.0) after controlling for several risk factors and other potential confounders. In subgroup analyses, elevated homocyst(e)ine levels appeared to be more strongly predictive of ischemic stroke in normotensive subjects and in men 60 years or younger. Although not statistically significant, in these subgroups increases in risks of 100% and 70%, respectively, were observed for men in the upper 20% of homocyst(e)ine values.

Conclusions In this study, the data were compatible with a small but nonsignificant association between elevated plasma homocyst(e)ine and risk of ischemic stroke. However, since the sample size is small and the confidence intervals are wide, either no association or a moderate increase in risk cannot be excluded, particularly in subgroups otherwise at low risk, eg, younger men and those with normal blood pressure. (***Stroke.* 1994;25:1924-1930.**)

Key Words • homocyst(e)ine • stroke, ischemic • prospective studies • blood pressure

Homocysteine is a thiol-containing amino acid derived from the metabolism of methionine. The term homocyst(e)ine is generally used to refer to homocysteine, disulfides of homocysteine with itself or with cysteine, and protein-bound forms.[1] Elevated homocyst(e)ine levels are observed in subjects with metabolic defects in several enzymes. These enzyme defects can be due to genetic disorders or deficiencies of folate, vitamin B_6, and vitamin B_{12}, essential cofactors in the metabolism of homocysteine.[2]

Several epidemiological studies, including a prospective study,[3] have shown that markedly elevated levels of homocyst(e)ine, either in the fasting state or after challenge with an oral dose of methionine, are associated with symptomatic atherosclerotic disease.[4,5] However, previous studies of occlusive cerebral disease were retrospective or cross sectional, and therefore the possibility could not be excluded that elevated plasma homocyst(e)ine was a consequence of the disease or conditions related to it.[6-13]

In a previous study, we observed that men with high levels of homocyst(e)ine were at a threefold higher risk of myocardial infarction.[3] In the present analysis, we examined the relation of homocyst(e)ine with risk of ischemic stroke in apparently healthy men 40 to 84 years old who were free from diagnosed stroke, transient ischemic attack, and myocardial infarction at baseline when they provided plasma samples.

Prior studies suggested that the relation between homocyst(e)ine levels and atherosclerotic disease might be influenced by the presence of hypertension. Thus, in patients with peripheral arterial occlusive diseases, hypertension was more prevalent in those with elevated homocyst(e)inemia.[14] Araki et al[6] concluded that high plasma levels of homocyst(e)ine in conjunction with hypertension in particular could increase the risk for atherosclerotic cerebral infarction. Similarly, a study showed a stronger association of plasma homocyst(e)ine and carotid artery intimal-wall thickening in hypertensives than in normotensives.[15] To address this matter, we analyzed the association between homocyst(e)ine level and ischemic stroke separately for men with and those without high blood pressure.

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Methods

Design and Study Population

We conducted a nested, case-control study in the Physicians' Health Study in which blood samples were collected prospectively. The Physicians' Health Study is an ongoing randomized, double-blind, placebo-controlled 2×2 factorial trial of aspirin and beta-carotene.[16,17] A total of 22 071 US male physicians who were 40 to 84 years old in 1982 were enrolled. Men were excluded if they had a prior history of stroke, transient ischemic attack, myocardial infarction, or cancer (except nonmelanoma skin cancer); current renal or liver disease, peptic ulcer, or gout; contraindication to aspirin; or current use of aspirin, other platelet-active agents, or vitamin A supplements. The aspirin component of the trial was terminated on January 25, 1988, principally because of a statistically extreme 44% reduction in first myocardial infarction among the aspirin-treated group.

At enrollment, study participants completed questionnaires concerning medical history, behavior, and use of medications and vitamin supplements. A limited food-frequency questionnaire, mainly focused on vitamin A and carotene, was also included. Before randomization, between August 1982 and December 1984, we sent kits for blood sampling to the participants, who were instructed to have their blood drawn into EDTA Vacutainer tubes, to centrifuge them, and to return the plasma in polypropylene cryopreservation vials by prepaid overnight courier. The kit included a cold pack to keep the specimens cool (but not frozen) until receipt at Channing Laboratory the next morning, when they were aliquoted and stored at −80°C. During storage, no specimen thawed or warmed substantially. We received specimens from 14 916 (68%) of the randomized physicians, more than 70% between September and November 1982. The study participants reported cerebrovascular and cardiovascular events and other acute illnesses annually. Persistent nonresponders to the questionnaires were contacted by telephone. Deaths were usually reported by the families or postal authorities. The follow-up was 99.7% complete for those with nonfatal outcomes and 100% complete for those with fatal outcomes. Strokes were confirmed by medical records if they were characterized by a typical neurological deficit that was sudden or rapid in onset, of at least 24 hours in duration, and attributable to a cerebrovascular event. The End Points Committee, consisting of four physicians who were blinded to treatment status, confirmed reported events by review of all medical records according to criteria established in the National Survey of Stroke.[18] The diagnosis was confirmed by computed tomography scans in 95% of patients with ischemic stroke. For fatal cases, we accepted diagnoses based on autopsy or confirmation by records that the death was due to cerebrovascular disease (International Classification of Diseases [ICD] codes 430-438).

Plasma homocyst(e)ine was measured in the samples from 109 subjects who were diagnosed with ischemic stroke (ICD code 434) and from 124 control subjects. Ninety-seven patients and control subjects were individually matched. Control subjects were free from any type of cerebrovascular disease at the time of the patient's diagnosis. They were randomly selected from participants who met the matching criteria of age (±1 year), smoking status (current, past, or never smoker), and length of follow-up (by 6-month intervals). To increase the statistical power of the study, we also performed unmatched analyses, using data from the total group of 109 subjects with ischemic stroke. The 12 additional patients had been matched to control subjects who had insufficient plasma for analysis. We also augmented the control group to 427 subjects by adding 303 control subjects from our recent study on homocyst(e)ine and risk of myocardial infarction.[3] In that study, control subjects had been matched to patients with myocardial infarction, using the same criteria as described above. Blood samples of patients with ischemic stroke and myocardial infarction and of all the control subjects were treated identically and were analyzed at the same time.

Laboratory Methods

Homocyst(e)ine levels were measured by Dr M. René Malinow at the Oregon Regional Primate Center. Homocyst(e)ine—the sum of homocysteine, the homocysteinyl moieties of homocysteine, and cysteine-homocysteine mixed disulfide, whether free or protein bound—was assayed by high-performance liquid chromatography and electrochemical detection, based on the method of Smolin and Schneider,[19] as previously described[20] with minor modifications.[21]

Samples from case-control pairs were handled together, blindly and identically throughout processing and analysis, and the position within pairs was varied randomly. Blind paired quality-control samples (n=26 pairs) were interspersed at random among the specimens. The quality-control samples were aliquots of a large, well-mixed plasma pool from healthy volunteers that were treated identically to the samples collected from the participants. The mean within-pair coefficient of variation in these paired quality control samples was 3.2%. Plasma levels of total and high-density lipoprotein (HDL) cholesterol were measured in the laboratory of Dr Frank Sacks.[22]

Statistical Analysis

We compared mean values and proportions of various cerebrovascular risk factors between patients and control subjects. For matched data, a paired Student's t test was used for the continuous variables and a McNemar paired χ^2 test was used for proportions, whereas for unmatched data, the unpaired Student's t test and a Pearson χ^2 test were used. Odds ratios and 95% confidence intervals were calculated for matched and unmatched data, using logistic regression. Conditional logistic regression (matched data) and multiple logistic regression (unmatched data) were used to control for several cerebrovascular risk factors simultaneously. Homocyst(e)ine levels above the 80th percentile among the control subjects were a priori defined as elevated levels, and those below the 80th percentile were defined as normal. Use of homocyst(e)ine concentrations above the 95th percentile as defining abnormal, as we did in our previous study,[3] would have led to unstable results in the matched analysis due to small numbers. However, for the unmatched data we show odds ratios for elevated homocyst(e)ine concentrations based on the 95th percentile cutoff point, to facilitate comparison with our earlier work. All P values are two-tailed. Associations between homocyst(e)ine levels and several risk factors for ischemic stroke were evaluated by calculating Spearman rank correlation coefficients. To evaluate a possible modifying role of high blood pressure, we performed separate risk analyses in strata of hypertensive and normotensive men, using unmatched data. High blood pressure was considered to be present in subjects with systolic blood pressure >150 mm Hg, diastolic blood pressure >90 mm Hg, or current use of antihypertensive drugs. This information was obtained through the enrollment questionnaire. Since elevated homocyst(e)ine levels mainly have been reported in subjects with premature vascular disease, we performed additional risk analyses in subgroups of men 60 years old or younger and men older than 60 years at the start of the study. To study whether the time of follow-up affected the association, we assessed the effect of elevated homocyst(e)ine levels among men stratified by the median time since enrollment in the trial, based on time of diagnosis for patients.

Results

Table 1 shows the risk factors of 109 men with ischemic stroke and 427 control subjects. Almost half of the patients had high blood pressure at enrollment

TABLE 1. Potential Risk Factors Among Patients With Ischemic Stroke and Control Subjects in the Physicians' Health Study

	Patients (n=109)	Control Subjects (n=427)	P*
Age, y	61.6±9.1	59.2±8.9	Matching variable†
Quetelet's index, weight in kg/(height in m)^2	26.0±3.7	24.9±2.8	.003
Systolic blood pressure, mm Hg	137±13	128±12	<.0001
Diastolic blood pressure, mm Hg	83±7	79±7	<.0001
Alcohol consumption, drinks/d	0.56±0.59	0.54±0.47	.81
Total cholesterol, mmol/L	206±37	214±36	.04
HDL cholesterol, mmol/L	45.6±12.9	49.5±13.1	.006
Ratio of total to HDL cholesterol	4.8±1.4	4.6±1.4	.13
With characteristic, %			
Cigarette smoking habits			
Never	39.5	40.8	Matching variable†
Past	38.5	41.8	
Current	22.0	17.4	
History of diabetes	12.8	3.0	<.0001
High blood pressure	46.8	19.2	<.0001

HDL indicates high-density lipoprotein. Values are mean±SD.
*Unpaired t test for continuous variables and χ^2 test for proportions.
†Differences in age and cigarette smoking between patients and control subjects were heavily influenced by matching for those variables, so tests for differences were not appropriate.

compared with one fifth of the control subjects. The proportion with history of diabetes was also higher among patients. The patients had a higher mean blood pressure and Quetelet's index, as well as lower total and HDL cholesterol levels. The total cholesterol–to–HDL cholesterol ratio and alcohol consumption were slightly, but not significantly, higher in patients than in control subjects. Control subjects were significantly younger than patients, due to the fact that the majority of the control subjects had originally been matched to men with myocardial infarction, whose mean age was lower than that of men with ischemic stroke. Cigarette smoking status, a matching variable, was identical among patients and control subjects.

We also compared the same risk factors among the 97 pairs of patients with ischemic stroke and matched control subjects. The mean levels and proportions of risk factors were similar to the ones observed in the unmatched groups. However, mean age and smoking habits did not differ, since these were matching factors (data not shown).

Overall, the mean level of homocyst(e)ine was slightly, although not statistically significantly, higher among patients with ischemic stroke (11.1±4.0 nmol/mL) than for control subjects (10.6±3.4 nmol/mL; P=.12). The mean homocyst(e)ine level for the matched patients was virtually the same as for the matched control subjects (Table 2).

The crude odds ratio of ischemic stroke, comparing individuals with elevated levels (higher than 80th percentile) with those with normal levels, was 1.4 (95% confidence interval [CI], 0.8 to 2.2). After adjustment for age, cigarette smoking habits, diabetes, high blood pressure, Quetelet's index, aspirin assignment, total cholesterol–to–HDL cholesterol ratio, and time since the last meal before the blood was drawn, the odds ratio was 1.2 (95% CI, 0.7 to 2.0) (Table 2). Five patients (5%) had plasma levels higher than the 95th percentile of the control distribution (16.6 nmol/mL), compared with 21 (5%) control subjects. Contrasting the top 5% with the lower 90% of the control distribution, we observed crude and multivariate adjusted odds ratios of 1.0 (95% CI, 0.4 to 2.7) and 0.8 (95% CI, 0.3 to 2.4), respectively (data not shown).

In a conditional logistic regression analysis in 97 patients with ischemic stroke and 97 control subjects matched for age and smoking status, both the crude and multivariate odds ratios revealed no effect of elevated homocyst(e)ine (Table 2).

We evaluated the associations of plasma homocyst(e)ine levels with several possible risk factors for ischemic stroke and other factors in control subjects (Table 3). The observed correlations were all small, although some of them were statistically significant. Blood pressure was directly associated with homocyst(e)ine levels, as were alcohol consumption and total cholesterol level. Plasma homocyst(e)ine levels correlated inversely, although not strongly, with multivitamin use (r=−.17; P=.0005). Most of the blood samples were not drawn from subjects in the fasting state. Among 351 control subjects we observed a positive association between homocyst(e)ine and time since the last meal before the blood was drawn (r=.14; P=.007). The mean amount of time that had elapsed since the last meal was 5 hours 52 minutes for patients and 5 hours 21 minutes for control subjects (P=.45) (data not shown).

To determine whether an association between homocyst(e)ine and risk of ischemic stroke was dependent on

TABLE 2. Homocyst(e)ine Levels and Risk of Ischemic Stroke

	Mean±SD, nmol/mL	*P*	Cutoff Points for Elevated Homocyst(e)ine Levels, nmol/mL	Patients, n	Control Subjects, n	Odds Ratio†
Unmatched analysis						
Patients (n=109)	11.1±4.0	.12*	>12.7‡	28	86	1.2 (95% CI, 0.7-2.0)
Control subjects (n=427)	10.6±3.4		≤12.7	81	341	
Matched analysis						
Patients (n=97)	11.1±4.2	.86*	>12.7‡	24	24	1.1 (95% CI, 0.5-2.8)
Control subjects (n=97)	11.0±4.8		≤12.7	73	73	

CI indicates confidence interval.

*Using an unpaired *t* test and a paired *t* test for unmatched and matched data, respectively.

†Adjusted for age, cigarette smoking status (either as matching factors or in multivariate logistic model), history of diabetes, high blood pressure, Quetelet's index, aspirin assignment, ratio of total cholesterol to high-density lipoprotein cholesterol, and hours since last meal.

‡80th percentile among 427 unmatched control subjects.

blood pressure, an interaction term was added to the multivariate model. The likelihood ratio test for the difference between the model with the interaction term and the model without it showed an improvement of the fit of the model when the interaction term was included ($P=.02$). We therefore separately analyzed subjects with normal and high blood pressure (Table 4). Among control subjects the mean homocyst(e)ine level was significantly higher in hypertensives than in normotensives, whereas among patients there was no such difference. Separate risk analyses for strata of hypertensive and normotensive men revealed that the impact of elevated homocyst(e)ine level was limited to normotensive subjects. In this group the multivariate adjusted odds ratio was 2.0 (95% CI, 1.0 to 4.0) for homocyst(e)ine concentrations >12.7 nmol/mL. The corresponding odds ratio in subjects with high blood pressure was 0.6 (95% CI, 0.3 to 1.5).

In an analysis restricted to men 60 years or younger at the start of the study (46 patients and 234 control subjects), the multivariate adjusted odds ratio was 1.7 (95% CI, 0.7 to 3.8) for subjects with elevated homocyst(e)ine levels compared with those with normal values. In men older than 60 years (63 patients and 193 control subjects) the corresponding odds ratio was 0.8 (95% CI, 0.4 to 1.8) (data not shown).

We further assessed the effect of elevated homocyst(e)ine levels, stratifying men by the median time since enrollment in the trial. The multivariate odds ratio for elevated homocyst(e)ine concentrations was 1.0 (96% CI, 0.5 to 2.4) when the analyses were restricted to patients who had been diagnosed before the median time. It was 1.3 (95% CI, 0.6 to 2.8) when only patients who had been diagnosed after that time were included in the analyses (data not shown).

TABLE 3. Spearman Correlation Coefficients Between Plasma Homocyst(e)ine Levels and Risk Factors in Control Subjects

Risk Factor	Correlation	*P*
Age	.01	.77
Quetelet's index	.09	.08
Systolic blood pressure	.12	.02
Diastolic blood pressure	.18	.0005
High blood pressure (yes versus no)	.18	.0002
History of diabetes (yes versus no)	.05	.31
Alcohol consumption	.12	.02
Multivitamin use	−.17	.0005
Total cholesterol	.10	.03
HDL cholesterol	−.06	.25
Ratio of total to HDL cholesterol	.11	.02

HDL indicates high-density lipoprotein.

The numbers for the correlations ranged from 383 to 427 because not all subjects had all measurements.

Discussion

In this prospective, nested case-control study, we observed a 20% higher risk of ischemic stroke in men with homocyst(e)ine values above the 80th percentile of the control distribution compared with those with lower values. This association was much weaker than that found in many previous studies on homocyst(e)ine and cerebrovascular disease. More convincing support for a potential role of homocyst(e)ine were the observed 100% and 70% increases in risk among normotensive men and men 60 years or younger, respectively. However, the findings in subgroups might be due to chance, especially considering the small number of subjects.

We considered several issues of internal validity that might explain why we observed a weaker association between homocyst(e)ine and occlusive cerebrovascular disease than did most other studies. Unlike previous investigations, our study used a prospective design, reducing the possibility that homocyst(e)ine levels were altered by the disease, medication, or changes in lifestyle associated with the disease. However, in a recent study, Malinow et al[15] studied the association between homocyst(e)ine and thickening of the carotid artery intimal-medial wall in asymptomatic individuals, a design less prone to bias than retrospective studies with symptomatic disease as an end point. The odds ratio for having a thickened carotid wall was 3.15 ($P=.001$) for subjects in the top quintile of plasma homocyst(e)ine level (>10.5 nmol/mL) compared with those in the

TABLE 4. Homocyst(e)ine Concentration and Risk of Ischemic Stroke in Strata of Normotensive and Hypertensive Subjects

	Odds Ratios for Elevated Concentrations of HCY*			
	Normotensive Subjects		Hypertensive Subjects	
	≤12.7 nmol/mL	>12.7 nmol/mL	≤12.7 nmol/mL	>12.7 nmol/mL
HCY level in patients†	11.1±4.4		11.3±3.5	
HCY level in control subjects†	10.3±3.3		11.8±3.6	
No. of patients/control subjects	42/288	16/57	39/53	12/29
Crude OR	1	1.9	1	0.6
Adjusted OR‡	1	2.0	1	0.6
95% confidence interval§		1.0-4.0		0.3-1.5

HCY indicates homocyst(e)ine; OR, odds ratio.
*Based on the distribution among 427 unmatched control subjects.
†Mean±SD nmol/mL.
‡Adjusted for age, cigarette smoking status, history of diabetes, Quetelet's index, aspirin assignment, ratio of total cholesterol to high-density lipoprotein cholesterol, and hours since last meal.
§For adjusted OR.

bottom quintile. Although the mean storage period in our study was about 8 years, since samples from patients and control subjects were stored for the same amount of time and were handled together and identically throughout processing, we do not consider this an important source of bias. Moreover, the mean value observed for control subjects was similar to those in studies using fresh samples. Most of our subjects were not fasting when the blood was drawn. It could be possible that fasting levels reveal abnormalities in homocyst(e)ine metabolism better, and thus use of nonfasting levels might have blurred the association. Furthermore, among control subjects we observed that subjects for whom the time since their last meal was longer tended to have higher plasma concentrations of homocyst(e)ine, a finding similar to that of Ubbink et al.[23] However, since patients had their blood drawn slightly longer after the last meal than did control subjects, this could only have caused their homocyst(e)ine concentrations to be higher, and therefore this cannot have been responsible for the observed weak association. Finally, we evaluated the likelihood of aspirin use being partly responsible for the observed weaker association. For subjects assigned to receive placebo, the mean homocyst(e)ine level was higher in patients than in control subjects, with a difference of 1.1 nmol/mL, compared with virtually no difference in the aspirin group. The multivariate adjusted odds ratio for subjects in the upper 20% compared with those in the bottom 80% of homocyst(e)ine levels was 1.3 (95% CI, 0.6 to 2.8) in the placebo group and 1.22 (95% CI, 0.8 to 1.6) in the aspirin group.

In our previous study of homocyst(e)ine and myocardial infarction in the same population, we showed a significant threefold relative risk for men with abnormally high homocyst(e)ine levels.[3] Blood samples from patients with MI and from patients with ischemic stroke were treated in the same way and were sent and analyzed at the same time under the same conditions. However, in the present study the number of ischemic strokes is modest, and the 95% CI therefore is rather wide.

In contrast to our findings, several studies have reported significantly higher fasting homocyst(e)ine levels in patients with cerebrovascular disease than in control subjects. A study by Araki et al[6] showed higher mean fasting levels of both free and total homocysteine among 45 patients with cerebral infarction than in 45 normotensive and 45 hypertensive control subjects of similar age and sex. Coull et al[12] reported a significantly higher mean fasting homocyst(e)ine concentration in 41 patients with acute stroke than in 31 control subjects. Recently, Brattström et al[10] detected fasting hyperhomocyst(e)inemia in 40% of 142 men and women with stroke but only in 4 of 66 control subjects. In that study, the cutoff point for hyperhomocyst(e)inemia among men was 17.7 nmol/mL. These results were consistent with previous findings of the same group of authors[8] but inconsistent with those of another of their studies, in which no significant difference in total fasting plasma homocyst(e)ine was found between 17 patients with premature cerebral thrombosis and 46 control subjects.[9] Mereau-Richard et al[13] found significantly higher fasting homocyst(e)ine levels in 92 patients with cerebral vascular disease before the age of 50 years, compared with those in 25 control subjects. Malinow et al[15] recently reported that fasting plasma levels of homocyst(e)ine were significantly higher in 287 subjects with thickened intimal-medial carotid walls than in 287 matched control subjects.

Several studies have reported data on plasma levels of homocyst(e)ine after methionine loading in relation to cerebrovascular risk.[7-9,11] All studies except one[9] showed a significantly higher proportion of subjects with hyperhomocyst(e)inemia (usually defined as exceeding the mean postload homocyst(e)ine level of control subjects by more than 2 SD) among patients with cerebrovascular disease than among control subjects.

In our study the mean age of patients at the time they were diagnosed with ischemic stroke was higher than in most of the other studies we mentioned above. Homocyst(e)ine levels have often been studied in patients with premature cerebrovascular disease, in most studies defined as first diagnosed before 50 or 55 years of age.[7-9,11,13] However, several studies found a positive association between homocyst(e)ine and vascular disease in subjects of the same age as in our study[6,10,12] or

even in older subjects.[10] We observed a stronger association between plasma homocyst(e)ine and risk of ischemic stroke among younger than among older men.

Considering that high blood pressure is a strong risk factor for ischemic stroke, one might expect to observe the highest relative risk associated with elevated levels of homocyst(e)ine in normotensive men. Indeed, we observed a stronger relation between homocyst(e)ine and risk of ischemic stroke in the group of normotensive subjects. The findings of Malinow et al,[15] who reported that the association between plasma homocyst(e)ine levels and risk for having a thickened carotid arterial wall was stronger among subjects with hypertension, contrast with our findings.

The association between high blood pressure and homocyst(e)ine levels that we observed in control subjects confirms previous observations (Levenson et al, personal communication). We considered that the use of antihypertensive drugs could have been responsible for the raised plasma homocyst(e)ine levels in hypertensives. However, homocyst(e)ine levels in 55 of the 82 control subjects (67%) with high blood pressure who were currently using antihypertensive drugs were not different than those in 26 subjects who had never used these drugs (1 subject had used antihypertensive drugs in the past). This finding is in concordance with that of Araki et al,[6] who found that significantly higher homocyst(e)ine levels in 45 normotensive compared with 45 hypertensive subjects could not be attributed to the use of antihypertensive drugs.

Many mechanisms for the observed positive association between homocyst(e)ine and vascular disease have been postulated. It has been suggested that the oxidation of homocysteine may result in formation of free radicals and hydrogen peroxide, promoting oxidation of low-density lipoprotein cholesterol[24,25] and damage of endothelial cells.[26]

Furthermore, findings in several in vitro studies suggest that homocysteine and its derivatives can affect blood coagulation factors by increasing platelet thromboxane production,[27] platelet aggregation,[28] and factor V activity[29] or by decreasing protein C activation.[30] Both the suggested atherogenic and thrombogenic effects of homocysteine, its derivatives, and related disulfides could account for a positive association between homocyst(e)ine and risk of ischemic stroke.

Increased plasma homocyst(e)ine levels might be the consequence of low plasma levels of vitamins B_6, B_{12}, and folate, since these are important cofactors in homocysteine metabolism.[31] We observed significant inverse associations for homocyst(e)ine levels with intakes of vitamin B_6, B_{12}, and folate.[3] However, these were themselves highly intercorrelated due to common sources of vitamin supplements and fortified foods. The correlations were most likely underestimates because the data on dietary intake were quite limited and incomplete. In the same population, plasma levels of vitamin B_6 and folate were highly inversely correlated with homocyst(e)ine levels (Chasan-Taber et al, submitted for publication). The nutritional state of physicians is presumably better than in the general population, which might explain why the difference between homocyst(e)ine levels for patients and control subjects was smaller than in other studies. In a recent cross-sectional study of Selhub et al[32] among elderly men and women, the prevalence of high homocyst(e)ine levels (>14 nmol/mL) was 29%, of which 67% could be attributed to inadequate plasma concentrations of one or more of the B vitamins involved in homocysteine metabolism.

In summary, the present study adds only weak evidence to the hypothesis that elevated homocyst(e)ine levels are an independent risk factor for ischemic stroke. In our population, the association appeared to be more pronounced among men who are at a low risk of having cerebrovascular disease, ie, young and without high blood pressure. We suggest that further prospective studies of homocyst(e)ine and risk of cerebrovascular disease be conducted, including, in particular, such persons at lower risk. Furthermore, research is needed to define which mechanisms can account for the observed positive relation between homocyst(e)ine levels and blood pressure. Sufficient data have accumulated to warrant clinical trials to evaluate the effect of lowering homocyst(e)ine levels for the primary and secondary prevention of vascular diseases.

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