Homocyst(e)ine and Risk of Cerebral Infarction in a Biracial Population : The Stroke Prevention in Young Women Study

Steven J. Kittner, Wayne H. Giles, Richard F. Macko, J. Richard Hebel, Marcella A. Wozniak, Robert J. Wityk, Paul D. Stolley, Barney J. Stern, Michael A. Sloan, Roger Sherwin, Thomas R. Price, Robert J. McCarter, Constance J. Johnson, Christopher J. Earley, David W. Buchholz and M. Rene Malinow

Stroke 1999;30;1554-1560
Stroke is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 0039-2499. Online ISSN: 1524-4628

Homocyst(e)ine and Risk of Cerebral Infarction in a Biracial Population

The Stroke Prevention in Young Women Study

Steven J. Kittner, MD, MPH; Wayne H. Giles, MD, MS; Richard F. Macko, MD; J. Richard Hebel, PhD; Marcella A. Wozniak, MD, PhD; Robert J. Wityk, MD; Paul D. Stolley, MD, MPH; Barney J. Stern, MD; Michael A. Sloan, MD; Roger Sherwin, MB, BChir; Thomas R. Price, MD; Robert J. McCarter, ScD; Constance J. Johnson, MD; Christopher J. Earley, MD, PhD; David W. Buchholz, MD; M. Rene Malinow, MD

Background and Purpose—Genetic enzyme variation and vitamin intake are important determinants of blood homocyst(e)ine levels. The prevalence of common genetic polymorphisms influencing homocyst(e)ine levels varies by race, and vitamin intake varies by socioeconomic status. Therefore, we examined the effect of vitamin intake, race, and socioeconomic status on the association of homocyst(e)ine with stroke risk.

Methods—All 59 hospitals in the greater Baltimore-Washington area participated in a population-based case-control study of stroke in young women. One hundred sixty-seven cases of first ischemic stroke among women aged 15 to 44 years were compared with 328 controls identified by random-digit dialing from the same region. Risk factor data were collected by standardized interview and nonfasting phlebotomy. Plasma homocyst(e)ine was measured by high-performance liquid chromatography and electrochemical detection.

Results—Blacks and whites did not differ in median homocyst(e)ine levels, nor did race modify the association between homocyst(e)ine and stroke. After adjustment for cigarettes per day, poverty status, and regular vitamin use, a plasma homocyst(e)ine level of $\geq$7.3 μmol/L was associated with an odds ratio for stroke of 1.6 (95% CI, 1.1 to 2.5).

Conclusions—The association between elevated homocyst(e)ine and stroke was independent not only of traditional vascular risk factors but also of vitamin use and poverty status. The degree of homocyst(e)ine elevation associated with an increased stroke risk in young women is lower than that previously reported for middle-aged men and the elderly and was highly prevalent, being present in one third of the control group. **(*Stroke*. 1999;30:1554-1560.)**

Key Words: case-control studies ■ cerebrovascular disorders ■ homocysteine ■ risk factors ■ vitamins

Homocyst(e)ine is produced from the essential amino acid methionine and metabolized via 3 pathways. It is catabolized to cysteine via the B_6-dependent enzyme cystathionine β-synthase, remethylated to methionine via the folic acid and B_{12}-dependent enzyme methionine synthase, or remethylated via the enzyme betaine homocysteine methyltransferase.[1] Thus, blood levels of homocyst(e)ine can be affected by genetic enzyme variation or vitamin intake; both factors are known to be important determinants of homocyst(e)ine levels in the population.[2,3]

The homocyst(e)ine theory of atherosclerosis was first promulgated by McCully[4] on the basis of a "natural experiment." He observed pathological findings in an infant with a rare inborn error of B_{12} metabolism similar to those in infants with cystathionine β-synthase deficiency. He concluded that extreme elevation in blood homocyst(e)ine, the only metabolic abnormality shared by these disorders, was the cause of the vascular disease in these children. Subsequently, a large body of observational epidemiological evidence, primarily case-control studies, linked even mild to moderate elevations in plasma homocyst(e)ine to vascular disease, including stroke, first among young adults and subsequently among persons of all ages.[5,6]

Nevertheless, relatively few population-based studies of the relationship between homocyst(e)ine and stroke risk have

Received February 18, 1999; final revision received April 13, 1999; accepted May 3, 1999.

From the Departments of Neurology (S.J.K, R.F.M., M.A.W.) and Epidemiology and Preventive Medicine (S.J.K., J.R.H., R.J.M., T.R.P., P.D.S.), University of Maryland at Baltimore; Geriatrics Research, Education, and Clinical Center, Baltimore Department of Veterans Affairs Medical Center (Md) (S.J.K., R.F.M); Department of Neurology, Johns Hopkins University, Baltimore, Md (D.W.B., C.J.E., C.J.J., R.J.W.); Division of Adult and Community Health, National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention, Atlanta, Ga (W.H.G.); Department of Neurology, Emory University, Atlanta, Ga (B.J.S.); Department of Neuroscience, Harbin Clinic, Rome, Ga (M.A.S.); Tulane School of Public Health and Tropical Medicine, New Orleans, La (R.S.); and Laboratory of Cardiovascular Diseases, Oregon Primate Research Center, Beaverton (M.R.M.).

Reprint requests to Dr Steven J. Kittner, Bressler Building, Room 12-006, University of Maryland at Baltimore, 655 W Baltimore St, Baltimore, MD 21201. E-mail skittner@umaryland.edu

***Stroke* is available at http://www.strokeaha.org**

been conducted in representative American populations that include minority groups. This is relevant for 2 reasons. First, vitamin intake varies with socioeconomic status. Second, the prevalence of common genetic polymorphisms, which result in impaired homocyst(e)ine metabolism, varies with race/ethnicity. Since low folic acid and vitamin B_6 intake is common among persons of lower socioeconomic status, including many blacks,[7,8] it is expected that moderate hyperhomocyst(e)inemia on a nutritional basis would be more prevalent among blacks. In contrast, the most common genetic polymorphism predisposing to elevated homocyst(e)ine is much more common among whites. The homozygous form of the Val MTHFR polymorphism[9] is associated with moderate hyperhomocyst(e)inemia when folic acid levels are low,[3] and its prevalence is estimated at 5% to 10% among whites and 1% among blacks.[10,11] By affecting the variation of homocyst(e)ine levels in different populations, these factors could influence the observed relationship of homocyst(e)ine to vascular disease events. Therefore, in the biracial population of the Baltimore-Washington corridor, we conducted a case-control study to examine the effect of vitamin intake, race, and socioeconomic status on the association of plasma homocyst(e)ine with stroke risk.

Subjects and Methods

The Stroke Prevention in Young Women Study is a population-based case-control study initiated to study risk factors for ischemic stroke in young women. The term *population-based* is taken to mean that both cases and their comparison group were identified from the same defined population. The study area included all of Maryland except the far western panhandle, Washington DC, and southern portions of both Pennsylvania and Delaware. Cases and controls were recruited to the study between February 26, 1992, and January 1, 1996. The study was approved by an institutional review committee, and the subjects gave informed consent.

Cases were women aged 15 to 44 years with a first cerebral infarction, identified by discharge surveillance at all 59 hospitals in the study area and through direct referral by regional neurologists. The methods for discharge surveillance, chart abstraction, case adjudication, and assignment of probable and possible underlying causes have been described previously.[12–14] Recruitment within 1 year of stroke was required for participation. Of 291 cases who were both eligible and identified within the 1-year time window after stroke, 227 could be contacted and agreed to participate; 171 had a blood sample obtained for homocyst(e)ine measurement. Of these, 4 were excluded because the blood sample was not processed within the 6-hour time limit, leaving a final sample of 167 cases.

Controls were women without a history of stroke, frequency-matched by age and geographic region of residence to the cases, identified by random-digit dialing. Of 450 eligible controls, 392 agreed to participate, and 335 had a blood sample obtained for homocyst(e)ine measurement. Of these, 7 were excluded because the blood sample was not processed within the 6-hour time limit, leaving a final sample of 328 controls.

Nonfasting blood samples for homocyst(e)ine were obtained at a median time after stroke of 87 days; 30.4% were obtained within 30 days of stroke. Samples for homocyst(e)ine were drawn into EDTA evacuated tubes (Vacutainer, Becton-Dickinson), immediately placed on ice, and transported to a central processing laboratory, where they were centrifuged at 1520*g* at 4°C for 15 minutes. Plasma homocyst(e)ine is known to be stable when whole blood is kept on ice for 6 hours before centrifugation.[16] Immediately after centrifugation, the plasma samples were placed in cryogenic vials and frozen at −70°C until they were shipped on dry ice to the Oregon Regional Primate Center for analysis. Total plasma homocyst(e)ine (the sum of free or protein-bound homocysteine, the homocysteinyl moieties of homocystine, and cysteine-homocysteine mixed disulfide) was determined by high-performance liquid chromatography and electrochemical detection as previously described.[17–19] Case and control samples were run batched in a 2:1 ratio, and laboratory personnel were blinded as to case-control status. Assays were run in duplicate, the results were averaged, and the analysis was repeated if duplicates differed 10% from the average. Standard curves were determined daily. The mean within-assay within-pair coefficient of variation (standard deviation, multiplied by 100 and divided by the mean) of this method is 3.2%.

Measured potential confounders and effect modifiers of the association between total plasma homocyst(e)ine and stroke included age, race, educational level, poverty status, hypertension, diabetes mellitus, angina or myocardial infarction, current smoking status, alcohol use, regular vitamin use, body mass index, total cholesterol, and HDL cholesterol. Hypertension, diabetes mellitus, and angina or myocardial infarction were determined by asking the study participant (or her proxy if the participant was unable to answer) if she had ever been told by a physician that she had the condition. Similarly, age, race, educational level, current smoking status, regular multivitamin use, alcohol use, and poverty status were determined by subject or proxy report. Current smokers were classified regarding the average number of cigarettes smoked per day, and regular multivitamin users were classified on the basis of the number of years of regular use during the prior 10 years. Poverty status, determined according to the 1993 Federal Poverty Income Guidelines,[20] was based on total family income and the number of household members. The poverty threshold was raised to 200% of that in the federal guidelines because of the high cost of living in Maryland and Washington, DC. Body mass index was based on self-report and calculated as the weight in kilograms divided by the square of the height in meters.

Total cholesterol and HDL cholesterol were measured according to standard practice,[21,22] and levels were considered high at 240 mg/dL and 35 mg/dL, respectively.

Wilcoxon rank sum tests were used to compare medians, and Fisher's exact tests were used to compare proportions. All probability values were 2-sided, and $P<0.05$ was considered statistically significant. Adjusted odds ratios (ORs) derived from logistic regression were used to determine whether homocyst(e)ine was associated with an increased risk for stroke after controlling for important confounders. A potential confounder was considered important if adjustment for that factor altered the unadjusted OR by ≥10%.[23] Effect modification was determined by including an interaction term in the logistic regression model.

Results

There were 167 cases included in the analyses. Those omitted were 60 participating patients for whom blood measurements for homocyst(e)ine were either not available or not processed within the 6-hour time window, as well as 33 eligible cases who refused to participate. Compared with the 167 cases included in the analysis, the 60 participants who did not have a homocyst(e)ine value did not have a statistically significant difference in medical record–derived age, race, hypertension, diabetes mellitus, current smoking status, or adjudicated stroke etiology. Compared with the 167 included cases, the 33 refusals were significantly more likely to be black (76% versus 44%), a current smoker (78% versus 45%), and diabetic (29% versus 13%).

Similarly, the 328 controls included in the analyses were compared with the 64 controls for whom blood measurements for homocyst(e)ine were either not available or not processed within the 6-hour time window, as well as with the 38 refusals for whom a brief telephone interview was available. There were no statistically significant differences in the questionnaire-

TABLE 1. Etiologies Among Cases With a Probable or Possible Cause of Stroke

	Probable Causes* (n=83)	Possible Causes† (n=32)
Large-artery atherosclerosis	15	10
Cardioembolism	16	18
Lacune	9	0
Other determined cause	43	4

*Four patients had 2 probable causes, but only 1 cause is listed per patient according to the following hierarchy: large-artery atherosclerosis>cardioembolism>lacune>other determined cause.

†Most patients had multiple possible causes, but only 1 cause is listed per patient according to the same hierarchy as for probable causes.

derived age, race, hypertension, diabetes mellitus, or current smoking status between the groups.

Stroke cases were classified as having a probable, possible, or undetermined etiology, as previously described.[12,13] Among the 167 stroke patients, 83 (50%) had at least 1 probable cause, 32 (19%) had no probable cause but at least 1 possible cause, and 52 (31%) were indeterminate. Table 1 shows the distribution of probable and possible causes. "Other determined causes" of stroke include hematologic disorders, nonatherosclerotic vasculopathy (eg, vasculitis and dissection), migraine, drug abuse, and stroke associated with the postpartum state.

Table 2 shows the median homocyst(e)ine level in the control group by selected categorical factors and the univariate relationship of these factors to case-control status. Some factors were associated with both homocyst(e)ine and stroke, while others were associated with only homocyst(e)ine or stroke. In this analysis, current cigarette smoking and total cholesterol were directly associated with both homocyst(e)ine and stroke, while regular vitamin use was inversely associated with both factors. Increased age was directly associated only with increased homocyst(e)ine. However, age was not associated with stroke because cases and controls were frequency matched for age. Factors associated only with increased stroke risk were black race, income below the poverty threshold, hypertension, coronary artery disease, diabetes mellitus, and higher body mass index and HDL cholesterol.

Table 3 shows the crude OR for stroke by quintile of homocyst(e)ine, with the first quintile serving as the reference category. Results are shown for the entire study population and stratified by race. Overall, there is no increase in stroke risk until the fourth quintile (homocyst(e)ine 7.30 to 9.39 μmol/L) (OR=2.0; 95% CI, 1.1 to 3.6) and no appreciable increased risk at the fifth quintile (OR=2.1; 95% CI, 1.2 to 3.8). This pattern was seen both among blacks and among whites and others. Therefore, to maximize power, subsequent analyses examine the risk of stroke associated with being in the higher 2 quintiles (high-homocyst(e)ine group) compared with the lower 3 quintiles (reference group) and do not stratify by race. Overall, 54% of cases and 33% of controls were in the high-homocyst(e)ine group.

Each covariate was screened for effect modification and confounding of the relationship between homocyst(e)ine and stroke. None of these factors modified the relationship, and only the number of cigarettes smoked per day, poverty status, and regular vitamin use were confounders.

Table 4 shows the results of logistic regression analysis in the total study population. The crude OR of 2.3 (95% CI, 1.6 to 3.4) for the high-homocyst(e)ine group was progressively reduced by the sequential addition to the model of cigarettes per day (OR=2.1; 95% CI, 1.4 to 3.1), poverty status (OR=1.8; 95% CI, 1.2 to 2.8), and lack of regular vitamin use (OR=1.6; 95% CI, 1.1 to 2.5). Nevertheless, even in the fully adjusted model, the OR for the high-homocyst(e)ine group remained statistically significant.

Discussion

In a large population-based case-control study among black and white women, we found that the association between moderate hyperhomocyst(e)inemia and stroke was independent not only of traditional vascular risk factors but also of vitamin use and poverty status. The magnitude of the increase in risk associated with elevated homocyst(e)ine (OR=1.6) was similar to that associated with smoking a pack of cigarettes per day (OR=1.9). Elevated homocyst(e)ine, as defined in this study, was highly prevalent, being present in one third of the control group

We found no evidence that the relationship between homocyst(e)ine and stroke is different for blacks than for the remainder of the study population, which was predominantly white. Furthermore, there was no evidence that homocyst(e)ine levels were higher in blacks than in whites (Table 2). In fact, the age-adjusted medians were 6.33 μmol/L for blacks and 6.40 μmol/L for whites (P=0.89). These findings regarding median homocyst(e)ine levels are consistent with national data among middle-aged and elderly persons from the National Health and Nutrition Examination Survey (NHANES).[24] In contrast, the Northern Manhattan Stroke Study[25] found that elderly blacks had higher homocyst(e)ine levels than whites, even after adjustment for age and other determinants of homocyst(e)ine level.

It is noteworthy that cigarette smoking was the only traditional vascular risk factor that required adjustment in our study. We also found it necessary to adjust for 2 other factors, current multivitamin use and poverty status, which have not been commonly included as potential confounders in other studies.[26,27] The regular use of multivitamins, which generally contain 0.4 mg of folic acid, 2 mg of B_6, and 6 μg of B_{12}, had a strong inverse association not only with homocyst(e)ine level but also with stroke risk. Poverty status had a weaker association with homocyst(e)ine levels, presumably on a dietary basis, but a strong association with stroke risk. Multivitamin use and poverty status had a strong association with stroke risk not only because of their effect on homocyst(e)ine but also because they are markers of other poorly defined health-affecting behaviors. The relationship of elevated homocyst(e)ine to stroke remained statistically significant, though attenuated in strength, after adjustment for these confounders.

Early prospective studies showed no[28] or only an equivocal[29] relationship of basal homocysteine to stroke risk. Recently, however, the British Regional Heart Study showed a

TABLE 2. Relationship of Selected Factors to Homocyst(e)ine Levels and Case-Control Status

Factor	Median* Homocyst(e)ine, μmd/L	*P*	Case Percentage	Control Percentage	*P*‡
Age, y		0.02			0.77
<40	6.07 (116)		37.1	35.4	
≥40	6.71 (212)		62.9	64.6	
Race		0.22			0.03
Black	6.65 (128)		47.9	39.0	
All others	6.15 (200)		51.5	61.0	
Educational level, y		0.90			1.00
≤11	6.47 (40)		12.2	12.2	
≥12	6.35 (288)		87.8	87.8	
Poverty		0.14			<0.01
Below line	6.52 (85)		44.5	26.8	
Above line	6.21 (232)		55.5	73.2	
Hypertension		0.40			<0.01
No	6.28 (286)		73.7	87.5	
Yes	6.83 (41)		26.4	12.5	
Coronary artery disease		0.19			<0.01
No	6.31 (318)		84.4	97.0	
Yes	7.41 (10)		15.6	3.1	
Diabetes		0.34			<0.01
No	6.42 (320)		87.4	97.9	
Yes	5.83 (7)		12.6	2.1	
Current smoking		<0.01			<0.01
No	6.11 (229)		54.8	69.8	
Yes	7.14 (99)		45.2	30.2	
Daily alcohol intake		0.23			0.06
Above median	6.16 (164)		40.7	50.0	
Below median	6.52 (164)		59.3	50.0	
Vitamin use		<0.01			<0.01
Yes	5.70 (194)		20.4	40.9	
No	6.88 (134)		79.6	59.2	
Current OC use		0.98			0.20
No	6.38 (285)		82.6	86.9	
Yes	6.15 (43)		17.4	13.1	
BMI		0.17			0.06
<27.2	6.18 (214)		55.7	65.2	
≥27.2	6.75 (114)		44.3	34.8	
Total cholesterol, mg/dL		0.02			<0.01
<240	6.25 (283)		74.1	88.2	
≥240	7.14 (38)		25.9	11.8	
HDL cholesterol, mg/dL		0.40			<0.01
≥35	5.54 (309)		5.0	5.5	
<35	6.43 (18)		85.0	94.5	
Lp(a)		0.61			0.06
Above median	6.56 (100)		54.4	50.3	
Below median	6.29 (99)		42.6	49.8	

OC indicates oral contraceptive; BMI, body mass index; and Lp(a), lipoprotein A.
*Controls only (n=328).
†Wilcoxon rank sum test.
‡Fisher's exact test.

TABLE 3. Crude ORs for Stroke of Homocyst(e)ine Quintiles in Entire Population and Stratified by Race

Homocyst(e)ine Quintiles, μmol/L	ORs and 95% CIs		
	Entire Population (n=495)	Blacks (n=179)	Whites and Others (n=315)
<5.03*	...	...	...
5.03–6.12	0.8 (0.4–1.5)	1.4 (0.5–4.0)	0.6 (0.3–1.3)
6.13–7.29	0.8 (0.4–1.6)	0.7 (0.3–2.1)	0.9 (0.4–1.9)
7.30–9.39	2.0 (1.1–3.6)	2.0 (0.8–5.3)	1.9 (0.9–3.9)
≥9.4	2.1 (1.2–3.8)	2.3 (0.9–6.4)	2.0 (0.9–4.1)

*Reference group.

strong, independent, and graded relationship of homocysteine to stroke risk during a 13-year follow-up study of men aged 40 to 59 years.[27] The contrasting results of these earlier cohort studies from the British Regional Heart Study may be due to underlying population differences, to inadequate power, or to differing procedures for collection of blood samples. It is known that homocyst(e)ine leaks out of red blood cells relatively rapidly at room temperature; the British study separated the serum from the blood samples within minutes of venipuncture. Our findings differ from the British results in several respects. Homocyst(e)ine values strongly increase with age and, to a lesser extent, with male sex; thus, the fourth quintile in our study would fall within the first or reference quartile of the British study. We did not observe a dose response; our results were more consistent with a threshold response. Unlike suggestions from the British Regional Heart Study[27] and the Atherosclerosis Risk in Communities Study,[30] we did not find definite evidence of an increased homocyst(e)ine effect in hypertensive subjects.

A potential criticism of this study is that, compared with cases who refused participation, the participating cases had a smaller proportion of blacks, diabetics, and cigarette smokers. This would tend to underestimate the effect of these factors on stroke risk but is unlikely to have substantially biased the OR for the association between homocyst(e)ine and stroke since race and diabetes were not strongly associated with homocyst(e)ine level and the number of cigarettes currently smoked per day was adjusted for in the analyses.

The present study has several limitations. The primary limitation is the fact that, in a case-control design, homocyst(e)ine levels are measured after the stroke. Thus, we cannot determine whether elevations in homocyst(e)ine were a precursor or a consequence of the stroke. Lindgren and colleagues[31] showed that homocyst(e)ine levels measured in the acute phase (mean, 2 days) after stroke are significantly lower than levels 1 to 2 years later, while no changes occurred in a control group. Similar findings were noted by Verhoef and coworkers[32] in regard to myocardial infarction. Egerton and colleagues[33] found that homocyst(e)ine is 25% lower on the first day after myocardial infarction compared with levels at 7 days or 6 months, changes that were inverse to those of C-reactive protein, an acute phase reactant. The findings from these studies are consistent but have an ambiguous interpretation, since they could be due to an acute decrease in homocyst(e)ine or to a poststroke chronic increase in homocyst(e)ine. Distinguishing between these 2 possibilities would require homocyst(e)ine measurement both before and after such an event. While prospective data show that homocyst(e)ine levels predict stroke in middle-aged men,[27] a prospective study design is not feasible in the young adult population because of the low incidence of stroke in this age range.

TABLE 4. OR for Stroke of the High-Hemocyst(e)ine Group Before and After Adjustment for Confounders

Covariants	OR	95% CI
None (crude)	2.3	1.6–3.4
Cigarettes per day	2.1	1.4–3.1
Cigarettes per day, poverty status	1.8	1.2–2.8
Cigarettes per day, poverty status, regular vitamin use	1.6	1.1–2.5

A second limitation of the study is that serum vitamin levels were not measured. Thus, we cannot determine whether there was an association of vitamin status with stroke and, if so, whether this association is entirely mediated by homocyst(e)ine. There is evidence from case-control[32,34] and prospective[35] data that low vitamin B_6 levels are associated with risk of vascular disease, independent of homocyst(e)ine level.

A third limitation of this study and all observational studies is that all exposures were measured with error. It could be argued[36] that inadequate measurement of other cardiovascular risk factors[37] or their inadequate control in statistical analyses could lead to bias in favor of finding an association between homocyst(e)ine and vascular disease. Certain important confounders, such as serum creatinine[27,38] and physical activity,[37] were not measured, and other factors, such as cholesterol, were measured subsequent to the event when cases would be more likely to have been treated. Conversely, blood samples for homocyst(e)ine were obtained in the nonfasting state under epidemiological field conditions. Had it been possible to reduce this measurement error or adjust for it, the observed relationships between homocyst(e)ine and stroke may have been stronger.

The potential for a causal relationship between moderate hyperhomocyst(e)inemia and vascular disease suggested by observational studies is bolstered by the biological underpinnings of the association. There is evidence that hyperhomocyst(e)inemia is both atherogenic[30,39,40] and prothrombotic,[41–46] operating through a variety of potential mechanisms including direct endothelial injury, mitogenic activity of vascular smooth muscle cells, impaired endogenous fibrinolysis, endothelial nitric oxide response, and inhibition of thrombomodulin-dependent protein C activation. Our findings in a young population in which atherosclerotic disease is rarely responsible for ischemic stroke[14,47] support the notion that homocyst(e)ine elevations are associated with a hypercoagulable state. The recent development of animal models for the pathological[48] and physiological[49] effects of hyperhomocyst(e)inemia provides further biological plausibility. Nevertheless, there is an important current of dissenting opinion regarding the importance of homocyst(e)ine elevations in the pathogenesis of vascular disease. This opinion is based primarily on criticisms of the applicability of

in vitro studies of the biological effects of homocyst(e)ine and a lack of consistency in the prospective studies of homocyst(e)ine and coronary heart disease.[50] Even prospective studies do not exclude the possible interpretation that vascular disease causes an increase in homocyst(e)ine levels, unless persons with subclinical vascular disease are excluded from the population at risk.[50] Intervention studies in animal models and humans are needed to determine which of the potential mechanisms of homocyst(e)ine-associated vascular risk are modifiable by targeted vitamin therapy. Most importantly, recently initiated primary and secondary prevention trials will determine whether lowering homocyst(e)ine with B vitamin interventions will lower risk for vascular disease events.

Appendix

The authors would like to acknowledge the assistance of the following individuals who have sponsored the Stroke Prevention in Young Women Study at their institution: Frank Anderson, MD; Clifford Andrew, MD, PhD; Christopher Bever, MD; Nicholas Buendia, MD; Young Ja Cho, MD; James Christensen, MD; Remzi Demir, MD; Terry Detrich, MD; John Eckholdt, MD; Nirmala Fernback, MD; Jerold Fleishman, MD; Benjamin Frishberg, MD; Stuart Goodman, MD, PhD; Norman Hershkowitz, MD, PhD; Luke Kao, MD, PhD; Mehrullah Khan, MD; Ramesh Khurana, MD; John Kurtzke, MD; William Leahy, MD; William Lightfoote II, MD; Bruce Lobar, MD; Micheal Miller, MD, PhD; Harshad Mody, MBBS; Marvin Mordes, MD; Seth Morgan, MD; Howard Moses, MD; Sivarama Nandipati, MD; Mark Ozer, MD; Roger Packer, MD; Thaddeus Pula, MD; Phillip Pulaski, MD; Naghbushan Rao, MD; Marc Raphaelson, MD; Solomon Robbins, MD; David Satinsky, MD; Elijah Saunders, MD; Micheal Sellman, MD, PhD; Arthur Siebens, MD (deceased); Harold Stevens, MD, PhD; Dean Tippett, MD; Roger Weir, MD; Micheal Weinrich, MD; Richard Weisman, MD; Don Wood, MD (deceased); and Mohammed Yaseen, MD.

In addition, the study could not have been completed without support from the administration and medical records staff at the following institutions: in Maryland: Anne Arundel Medical Center; Atlantic General Hospital; Bon Secours Hospital; Calvert Memorial Hospital; Carroll County General; Church Hospital Corporation; Doctors Community Hospital; Fallston General Hospital; Franklin Square Hospital Center; Frederick Memorial Hospital; The Good Samaritan Hospital of Maryland, Inc; Greater Baltimore Medical Center; Harbor Hospital Center; Hartford Memorial Hospital; Holy Cross Hospital; Johns Hopkins Bayview, Inc; Johns Hopkins Hospital; Howard County General Hospital, Inc; Kennedy Krieger Institute; Kent and Queen Anne Hospital; Laurel Regional Hospital; Liberty Medical Center, Inc; Maryland General Hospital; McCready Memorial Hospital; Memorial Hospital at Easton; Mercy Medical Center; Montebello Rehabilitation Hospital; Montgomery General Hospital; North Arundel Hospital; Northwest Hospital Center; Peninsula Regional Medical Center; Physician's Memorial Hospital, Prince George's Hospital Center; Saint Agnes Hospital; Saint Joseph Hospital; Saint Mary's Hospital; Shady Grove Adventist Hospital; Sinai Hospital of Baltimore; Southern Maryland Hospital Center; Suburban Hospital; The Union Memorial Hospital; University of Maryland Medical System; Department of Veterans Affairs Medical Center in Baltimore; Washington Adventist Hospital; and Washington County Hospital; in Washington DC: Children's National Medical Center; District of Columbia General Hospital; The George Washington University Medical Center; Georgetown University Hospital; Greater Southeast Community Hospital; Hadley Memorial Hospital; Howard University Hospital; National Rehabilitation Hospital; Providence Hospital; Sibley Memorial Hospital; Veterans Affairs Medical Center; and the Washington Hospital Center; in Pennsylvania: Gettysburg Hospital and Hanover General Hospital.

Acknowledgments

This study was supported by a clinical stroke research center award (NS16332-11) from the National Institute of Neurological Disorders and Stroke. Dr Kittner was supported in part by the Geriatric Research, Education, and Clinical Center at the Baltimore Veterans Affairs Medical Center. We are indebted to Barbara Upson for her outstanding technical assistance and to the following members of the Stroke Prevention in Young Women research team for their dedication: Anne Epstein, Barbara Feeser, James Gardner, Mary Keiser, Ann Maher, Jennifer Rohr, Mary J. Seipp, Susan Snyder, Mary J. Sparks, and Nancy Zappala.

References

1. Mudd SH, Levy HL, Skovby F. Disorders of transsulfuration. In: Scriver CR, Beaudet AL, Slly WS, Valle D, eds. *The Metabolic and Molecular Basis of Inherited Disease*. Vol 1. 7th ed. New York, NY: McGraw-Hill Inc; 1995:1279–1327.
2. Selhub J, Jacques PF, Wilson PWF, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocystinemia in an elderly population. *JAMA*. 1993;270:2693–2698.
3. Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, Selhub J, Rozen R. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine metabolism. *Circulation*. 1996;93:7–9.
4. McCully KS. Vascular pathology of homocystinemia: implications for the pathogenesis of atherosclerosis. *Am J Pathol*. 1969;56:111–128.
5. Boushey CJ, Beresford SAA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. *JAMA*. 1995;274:1049–1057.
6. Kittner SJ. Homocysteine and ischemic stroke [in French]. *Sang Thrombose Vaisseaux*. 1996;8:603–606.
7. Subar AF, Block G, James LD. Folate intake and food sources in the US population. *Am J Clin Nutr*. 1989;50:508–516.
8. Manore MM, Vaughan LA, Carroll SS, Leklem JE. Plasma pyridoxal 5′-phosphate concentration and dietary vitamin B-6 intake in free-living, low-income elderly people. *Am J Clin Nutr*. 1989;50:339–345.
9. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP, Rozen R. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet*. 1995;10:111–113.
10. Stevenson RE, Schwartz CE, Du YZ, Adams MJ Jr. Differences in methylenetetrahydrofolate reductase genotype frequencies, between whites and blacks. *Am J Hum Genet*. 1997;60:229–230.
11. Giles WH, Kittner SJ, Ou CY, Croft JB, Brown V, Buchholz DW, Earley CJ, Feeser BR, Johnson CJ, Macko RF, McCarter RJ, Price TR, Sloan MA, Stern BJ, Wityk RJ, Wozniak MA, Stolley PD. Thermolabile ethylenetetrahydrofolate reductase polymorphism (C677T) and total homocysteine concentration among African-American and white women. *Ethn Dis*. 1998;8:149–157.
12. Johnson CJ, Kittner SJ, McCarter RJ, Sloan MA, Stern BJ, Buchholz D, Price TR. Interrater reliability of an etiologic classification of ischemic stroke. *Stroke*. 1995;26:46–51.
13. Kittner SJ, Stern BJ, Feeser BR, Hebel R, Nagey DA, Buchholz DW, Earley CJ, Johnson CJ, Macko RF, Sloan MA, Wityk RJ, Wozniak MA. Pregnancy and the risk of stroke. *N Engl J Med*. 1996;335:768–774.
14. Kittner SJ, Stern BJ, Wozniak M, Buchholz DW, Earley CJ, Feeser BR, Johnson CJ, Macko RF, McCarter RJ, Price TR, Sherwin R, Sloan MA, Wityk RJ. Cerebral infarction in young adults: the Baltimore-Washington Cooperative Young Stroke Study. *Neurology*. 1998;50:890–894.
15. Deleted in proof.
16. Kittner SJ, Malinow MR, Seipp MJ, Upson B, Hebel JR. Stability of blood homocyst(e)ine under epidemiological field conditions. *J Clin Lab Anal*. 1995;9:75–76.
17. Smolin LA, Schneider JA. Measurement of total plasma cysteamine using high-performance liquid chromatography with electrochemical detection. *Anal Biochem*. 1988;168:374–379.
18. Malinow MR, Kang SS, Taylor LM, Wong PW, Coull B, Inahara T, Mukerjee D, Sexton G, Upson B. Prevalence of hyperhomocyst(e)inemia in patients with peripheral arterial occlusive disease. *Circulation*. 1989; 79:1180–1188.
19. Malinow MR, Sexton G, Averbuch M, Grossman M, Wilson D, Upson B. Homocyst(e)inemia in daily practice: levels in coronary artery disease. *Coron Artery Dis*. 1990;1:215–220.

20. *Federal Poverty Income Guidelines*. Washington, DC: Federal Register; February 12, 1993. Vol 58, No. 28.
21. Tietz NW, ed. *Fundamentals of Clinical Chemistry*. Philadelphia, Pa: WB Saunders Co; 1976.
22. Warnick G, Russell J, Albers JJ. Dextran sulfate-Mg2+ precipitation procedures for quantification of high density-lipoprotein-cholesterol. In: Copper GR, ed. *Selected Methods of Clinical Chemistry*. Vol 10. Washington, DC: American Association for Clinical Chemistry; 1983:91–99.
23. Maldonada G, Greenland S. Simulation study of confounder-selection strategies. *Am J Epidemiol*. 1993;138:923–936.
24. Giles WH, Croft JB, Greenlund KJ, Ford ES, Kittner SJ. Total homocyst(e)ine concentration and the likelihood of nonfatal stroke: results from the Third National Health and Nutrition Examination Survey, 1988–1994. *Stroke*. 1998;29:2473–2477 .
25. Jacobs BR, Sacco RL, Roberts JK, Chen X, Paik MC, Stabler SP. Race-ethnicity and other environmental determinants of serum homocysteine in northern Manhattan. *Stroke*. 1998;29:227. Abstract.
26. Graham IM, Daly LE, Refsum HM, Robinson K, Brattstrom LE, Ueland PM, Palma-Reis RJ, Boers GH, Sheahan RG, Israelsson B, Uiterwaal CS, Meleady R, McMaster D, Verhoef P, Witteman J, Rubba P, Bellet H, Wautrecht JC, de Valk HW, Sales Luis AC, Parrot-Rouland FM, Tan KS, Higgins I, Garcon D, Medrano MJ, Candito M, Evans AE, Andria G. Plasma homocysteine as a risk factor for vascular disease: the European Concerted Action Project. *JAMA*. 1997;277:1775–1781.
27. Perry IF, Refsum H, Morris RW, Ebrahim SB, Ueland PM, Shaper AG. Prospective study of serum total homocysteine concentration risk of stroke in middle-aged British men. *Lancet*. 1995;346:1395–1398.
28. Alfthan G, Pekkanen J, Jauhiainen M, Pitkaniemi J, Karvonen M, Tuomilehto J, Salonen JT, Ehnholm C. Relation of serum homocysteine and lipoprotein(a) concentrations to atherosclerotic disease in a prospective Finnish population based study. *Atherosclerosis*. 1994; 106:9–19.
29. Verhoef P, Hennekens CH, Malinow MR, Kok FJ, Willet WC, Stampfer MJ. A prospective study of plasma homocyst(e)ine and risk of ischemic stroke. *Stroke*. 1994;25:1924–1930.
30. Malinow MR, Nieto FJ, Szklo M, Chambless LE, Bond G. Carotid artery intimal-medial wall thickening and plasma homocyst(e)ine in asymptomatic adults: the Atherosclerosis Risk in Communities Study. *Circulation*. 1993;87:1107–1113.
31. Lindgren A, Brattstrom L, Norrving B, Hultberg B, Andersson A, Johannson BB. Plasma homocysteine in the acute and convalescent phases after stroke. *Stroke*. 1995;26:795–800.
32. Verhoef P, Stampfer MJ, Buring JE, Gaziano JM, Allen RH, Stabler SP, Reynolds RD, Kok FJ, Hennekens CH, Willett WC. Homocysteine metabolism and risk of myocardial infarction: relation with vitamins B6, B12, and folate. *Am J Epidemiol*. 1996;1;143:845–859.
33. Egerton W, Silberberg J, Crooks R, Ray C, Xie L, Dudman N. Serial measures of plasma homocyst(e)ine after acute myocardial infarction. *Am J Cardiol*. 1996;77:759–761.
34. Robinson K, Arheart K, Refsum H, Brattstrom L, Boers G, Ueland P, Rubba P, Palma-Reis R, Meleady R, Daly L, Witteman J, Graham I, for the European COMAC Group. Low circulating folate and vitamin B6 concentrations: risk factors for stroke, peripheral vascular disease, and coronary artery disease. *Circulation*. 1998;97:437–443.
35. Folsom AR, Nieto FJ, McGovern PG, Tsai MY, Malinow MR, Eckfeldt JH, Hess DL, Davis CE. Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related genetic polymorphisms, and B vitamins: the Atherosclerosis Risk in Communities (ARIC) Study. *Circulation*. 1998;98:204–210.
36. Evans RW, Shaten BJ, Hempel JD, Cutler JA, Kuller LH. Homocyst(e)ine and risk of cardiovascular disease in the Multiple Risk Factor Intervention Trial. *Arterioscler Thromb Vasc Biol*. 1997;17:1947–1953.
37. Nygard O. Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, Ueland PM, Kvale G. Total plasma homocysteine and cardiovascular risk profile: the Hordaland Homocysteine Study. *JAMA*. 1995;274: 1526–1533.
38. Coull BM, Malinow MR, Beamer N, Sexton G, Nordt F, de Garmo P. Elevated plasma homocyst(e)ine concentration as a possible independent risk factor for stroke. *Stroke*. 1990;21:572–576.
39. Tsai JC, Perrella MA, Yoshizumi M, Hsieh CM, Haber E, Schlegel R, Lee ME. Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis. *Proc Natl Acad Sci U S A*. 1994;91: 6369–6373.
40. Selhub J, Jacques PF, Bostom AG, D'Agostino RB, Wilson PWF, Belanger AJ, O'Leary DH, Wolf PA, Schaefer EJ, Rosenberg IH. Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. *N Engl J Med*. 1995;332:286–291.
41. Lentz SR, Sadler JE. Inhibition of thrombomodulin surface expression and protein C activation by the thrombogenic agent homocysteine. *J Clin Invest*. 1991;88:1906–1914.
42. Stamler JS, Osborne JA, Jaraki O, Rabbani LE, Mullins M, Singel D, Loscalzo J. Adverse vascular effects of homocysteine are modulated by endothelial-derived relaxing factor and related oxides of nitrogen. *J Clin Invest*. 1993;91:308–318.
43. Bienvenu T, Ankri A, Chadefaux B, Montalescot G, Kamoun P. Elevated total plasma homocysteine, a risk factor for thrombosis: relation to coagulation and fibrinolytic parameters. *Thromb Res*. 1993;70:123–129.
44. Falcon CR, Cattaneo M, Panzeri D, Martinelli I, Mannucci PM. High prevalence of hyperhomocyst(e)inemia in patients with juvenile venous thrombosis. *Arterioscler Thromb*. 1994;14:1080–1083.
45. Schreiner PJ, Wu KK, Malinow MR, Nieto FJ, Heiss G. Plasma homocyst(e)ine and hemostasis: the ARIC Study. *Circulation*. 1994;89:11. Abstract.
46. Van den berg M, Boers GHJ, Franken DG, Blom HJ, Van Kamp GJ, Jakobs C, Rauwerda JA, Kluft C, Stehouwert CDA. Hyerhomocysteinemia and endothelial dysfunction in young patients with peripheral arterial occlusive disease. *Eur J Clin Invest*. 1995;25:176–181.
47. Adams HP Jr, Kappelle LJ, Biller J, Gordon DL, Love BB, Gomez F, Heffner M. Ischemic stroke in young adults: experience in 329 patients enrolled in the Iowa Registry of stroke in young adults. *Arch Neurol*. 1995;52:491–495.
48. Rolland PH, Friggi A, Barlatier A, Piquet P, Latrille V, Faye MM, Guillou J, Charpiot P, Bodard H, Ghiringhelli O, Calaf R, Luccioni R, Garcon D. Hyperhomocysteinemia-induced vascular damage in the minipig: captopril-hydrochlorothiazide combination prevents elastic alterations. *Circulation*. 1995;91:1161–1174.
49. Lentz SR, Malinow MR, Piegors DJ, Bhopatkar-Teredesai M, Faraci FM, Heistad DD. Consequences of hyperhomocyst(e)inemia on vascular function in atherosclerotic monkeys. *Arterioscler Thromb Vasc Biol*. 1997;17:2930–2934.
50. Kuller LH, Evans RW. Homocysteine, vitamins, and cardiovascular disease. *Circulation*. 1998;98:196–199.