Low Vitamin B6 but Not Homocyst(e)ine Is Associated With Increased Risk of Stroke and Transient Ischemic Attack in the Era of Folic Acid Grain Fortification

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Background and Purpose—The introduction of cereal grain folic acid fortification in 1998 has reduced homocyst(e)ine (tHcy) concentrations in the US population. We performed a case-control study to determine the risk of stroke and transient ischemic attack (TIA) associated with tHcy and low vitamin status in a postfortification US sample.

Methods—Consecutive cases with new ischemic stroke/TIA were compared with matched controls. Fasting tHcy, folate, pyridoxal 5′-phosphate (PLP), B12, and MTHFR 677C>T genotype were measured.

Results—Mean PLP was significantly lower in cases than controls (39.97 versus 84.1 nmol/L, P<0.0001). After stroke risk factors were controlled for, a strong independent association was present between stroke/TIA and low PLP (adjusted odds ratio [OR], 4.6; 95% CI, 1.4 to 15.1; P<0.001) but not elevated tHcy (OR, 0.92; 95% CI, 0.4 to 2.1).

Conclusions—Low B6 but not tHcy was strongly associated with cerebrovascular disease in this postfortification, folate-replete sample. (Stroke. 2003;34:e51-e54.)

Key Words: cerebrovascular disorders ■ homocyst(e)ine ■ pyridoxine ■ risk factors

Extensive interest has been directed toward the role of elevated homocyst(e)ine (tHcy) as a candidate risk factor for vascular disease and ischemic stroke. Low folate, B12, and pyridoxine (B6) and a common genetic polymorphism (MTHFR 677C>T) are commonly associated with elevated tHcy, because these factors play key roles in homocysteine degradation pathways. Prospective studies that measured tHcy before stroke onset have not consistently confirmed the robust association reported by earlier case-control studies, raising doubts about the degree of risk associated with tHcy. One possible explanation for these discrepant results is that elevated tHcy in patients with vascular disease may be a marker of lower vitamin intake or reduced renal elimination resulting from subclinical atherosclerosis.

Since early descriptions of atherosclerosis in pyridoxine-deficient monkeys, several studies have reported an association between vascular disease and low B6 and folate. Studies in the United States that have examined the risk of stroke associated with tHcy have sampled the population before the introduction of cereal grain folic acid fortification in 1998. If the association between tHcy and stroke is mediated via low vitamin status, it is important to reexamine this relationship in the era of widespread folic acid supplementation in the United States.

Exclusion criteria for both cases and controls were (1) prespecified nonatherosclerotic stroke syndromes (hemorrhage, endocarditis, vasculitis, migraine), (2) diseases or medications affecting folate or homocysteine metabolism (cirrhosis, leukemia, psoriasis, dialysis, phenytoin, methotrexate), and (3) inability to obtain consent. Potential controls with prior stroke, TIA, or carotid endarterectomy were also excluded.

Stroke severity was measured by the admission National Institutes of Health Stroke Scale (NIHSS) score. Stroke subtype was assigned by a blinded stroke neurologist according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria.

Biochemical/Genetic Assays
Fasting tHcy was measured by florescence polarization immune assay (coefficient of variation [CV], 3.7% to 5.2%). Serum folate
(CV, 10.9%), B12 (CV, 7.7%), and red cell folate (CV, 10%) were measured by immunoassay (Elecsys 2010, Roche). Pyridoxal 5'-phosphate (PLP) was measured by the tyrosine decarboxylation method (CV, 22%). MTHFR genotype was measured by Hinfl restriction digestion. A value of 11.4 μmol/L (the value above which tHcy was associated with carotid stenosis in the Framingham cohort) was chosen to define elevated tHcy, because no threshold value exists for the association with vascular disease. Low vitamin levels were defined as: low folate, 7 nmol/L (3 ng/mL); low B12, 185 pmol/L (250 pg/mL); and low PLP, 20 nmol/L.

Statistical Analysis

Log transformations were used for analyses of skewed data (tHcy and PLP). Univariate (2-sided t tests and 2 tests, Pearson’s correlation) and multivariable (multiple logistic and linear regression) analyses were performed. Odds ratios (ORs) and 95% CIs were calculated by standard formulas. To determine PLP quintile categories, individual PLP values were combined, and the threshold values defining each quintile were calculated.

Results

Baseline Characteristics

Three hundred twenty subjects (180 cases, 140 controls) were included (Table 1). One hundred seventy-one cases (95%) had ischemic stroke, and 9 (5%) had TIA caused by large-artery disease. There was no difference between groups in age, sex, and supplemental vitamin use. Of cases, 75% had phlebotomy within 5 days of symptom onset (median, 4 days).

**tHcy and Risk of Cerebrovascular Disease**

Mean tHcy among cases did not differ from that in controls (Table 1). The adjusted OR for stroke/TIA associated with elevated tHcy was 0.92 (95% CI, 0.4 to 2.1; P=0.8).

On univariate analysis, age (r=0.17, P=0.004), PLP (r=-0.15, P=0.01), B12 (r=-0.24, P<0.0001), serum folate, and vitamin B6/B12 were each associated with stroke/TIA (Table 2). Multivariable analysis showed significant associations of stroke/TIA with age (OR=1.15, P=0.02), PLP (OR=6.32, P<0.0001), folate (OR=5.16, P=0.0007), vitamin B6/B12 (OR=6.08, P=0.0002), and hypertension (OR=11.01, P=0.0001). The adjusted OR for stroke/TIA associated with elevated tHcy was 0.57 (95% CI, 0.33 to 0.97; P=0.04) in those on vitamin supplementation and 0.91 (95% CI, 0.4 to 2.2; P=0.9) in those not on vitamin supplementation.
related to major nutritional and genetic determinants of folate \( (P=0.0001) \) (Table 1). Low PLP \( (<20 \text{ nmol/L}) \) was significantly lower in cases than in controls \( (P<0.001) \). A highly significant, graded inverse correlation was present \( (r=-0.12, P=0.03) \), creatinine \( (r=0.34, P<0.0001) \), NIHSS \( (r=-0.23, P=0.002) \), hypertension \( (P=0.008) \), MTHFR 677 TT genotype \( (P=0.09) \), and vitamin use \( (P<0.001) \) were associated with tHcy. After adjustment for multiple variables, B12 \( (P=0.02) \), creatinine \( (P=0.05) \), and NIHSS \( (P=0.059) \) remained independent predictors of tHcy.

Although mean tHcy was slightly higher in the patients with large-artery stroke, no significant differences in tHcy were present among stroke subtypes.

Vitamin Levels and Risk of Cerebrovascular Disease

PLP was significantly lower in cases than in controls \( (P<0.0001) \) (Table 1). Low PLP \( (<20 \text{ nmol/L}) \) was strongly associated with stroke/TIA after adjustment for vascular risk factors, vitamin supplement use, and tHcy (OR, 4.6; 95% CI, 1.4 to 15.1; \( P<0.001) \). A highly significant, graded inverse association was present between PLP and stroke \( (P<0.001 \text{ for trend}) \) (the Figure), with a protective influence observed with higher PLP levels. After adjustment for stroke risk factors, tHcy, and creatinine, the OR of stroke/TIA for the fourth compared with lowest PLP quintile was 0.14 (95% CI, 0.04 to 0.52; \( P=0.003) \), whereas the OR for those in the fifth compared with lowest quintile was 0.1 (95% CI, 0.03 to 0.38; \( P<0.001) \).

To examine for the possibility that the association might be due to a stroke-induced decline in albumin (which binds PLP in vivo), we repeated the analyses with albumin as a covariate in the model. The relationship between PLP and stroke remained unchanged. To further examine this question, we investigated posthoc for a correlation between PLP and the onset-phlebotomy interval, which might be suggestive of a progressive stroke-induced PLP decline. No evidence of a correlation was present \( (r=0.04, P=0.6) \).

Serum folate \( (P=0.05) \) and B12 \( (P=0.03) \) were lower in cases than controls (Table 1). After adjustment for vascular risk factors and tHcy, no association was observed between B12 or folate and stroke/TIA.

Discussion

Our study describes the risk of stroke and large-artery TIA related to major nutritional and genetic determinants of plasma tHcy in subjects studied after the introduction of folic acid fortification in the United States. We found a strong inverse association between stroke/TIA and B6 status that was independent of other vascular risk factors and tHcy, suggesting that it was mediated via mechanisms other than elevated tHcy. In contrast to several US studies performed before folic acid fortification, we found no difference in tHcy between subjects with cerebrovascular disease and controls.

It is important to consider the possible effects of stroke on tHcy when interpreting our finding of similar tHcy in cases and controls. Howard et al\(^8\) have reported that tHcy rises by 6% to 10% within the first 5 days after ischemic stroke and is elevated in the convalescent compared with the acute phase. When tHcy is measured after stroke, this increases the likelihood of overestimation of baseline tHcy, leading to a false-positive association. Because 75% of cases were sampled within 5 days in our study, it is highly unlikely that a clinically meaningful overestimate of tHcy occurred. This is supported by the similar tHcy distributions between groups, a finding that argues against a measurement bias favoring higher tHcy in cases.

Data from the Framingham cohort indicate that mean tHcy and the prevalence of hyperhomocysteinemia have fallen since the introduction of folic acid grain fortification.\(^9\) One plausible explanation of our finding is that additional folic acid intake since fortification may have disproportionately reduced tHcy among individuals with higher prefortification levels, thus eliminating the difference between subjects with and without vascular disease seen in earlier observational studies.

To the best of our knowledge, only 2 prior studies have examined the risk of stroke and PLP, both of which were performed in non-US populations.\(^4,5\) As in our study, studies of coronary disease have found that the association remained after adjustment for tHcy, suggesting that the association may be mediated via mechanisms other than impaired homocysteine transsulfuration.\(^1,3–6\) In our study, selection bias is unlikely to explain this result, because the groups were almost identical in age and vitamin supplementation use, 2 major determinants of B6 status.

Although the case-control design prevents a definitive conclusion regarding prestroke vitamin status, it is possible that the difference in PLP between groups reflects lower B6 status in cases preceding the stroke. As is inherent in the case-control design, it is also possible that PLP may have been affected by some factor related to the stroke event. We consider it unlikely that low PLP in cases was due to a decline in albumin, because the association remained after adjustment for albumin level. We also believe it unlikely that acute poststroke nutritional changes explain the findings, because plasma PLP changes slowly in response to reduced intake as a result of sustained release from tissue stores.\(^7\) One mechanism by which PLP might change after acute stroke relates to altered metabolism caused by acute-phase inflammation. Growing evidence suggests that B6 status may be closely related to acute and chronic inflammation. In ambulatory elderly subjects from the Framingham cohort, PLP was strongly inversely associated with C-reactive protein, an inflammatory marker associated with atherosclerosis.\(^10\) Re-
cent studies have also reported strong inverse correlations between PLP and inflammatory markers in rheumatoid arthritis, a prototypic inflammatory disease. If verified, this inflammation-related mechanism may partially explain the findings of previous prospective and case-control studies linking low B6 status to vascular disease.

Acknowledgments

Dr Kelly is currently the recipient of a Clinical Scientist Development Award from the Doris Duke Charitable Foundation. During the period that this work was performed, he received funding from the American Stroke Association and was a member of the Clinical Investigator Training Program, Harvard/MIT Health Sciences and Technology–Beth Israel Deaconess Medical Center, in collaboration with Pfizer Inc. We are grateful to the following sources of endowed support for clinical research in stroke and metabolic disorders: the Esther U. Sharp, Theodore Levitt, Paul Davin, John Larson, and Mary L. Efron funds; the Schoolman Stroke Prevention Research Fund; and the John Conway and Merrill Lynch Stroke Fellowship endowments. We thank the patients who participated and Brenda Thornell, Joan O’Donnell, Sharon Silveira, and Sam Phinney for their assistance.

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*Stroke*. 2003;34:e51-e54; originally published online May 8, 2003; doi: 10.1161/01.STR.0000071109.23410.AB
*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/34/6/e51

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