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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.

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(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 mol/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 χ² 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...
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. 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. One plausible explanation of our finding is that additional folic acid intake since fortification may have disproportionately reduced tHcy among individuals with higher pref fortification 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. 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. 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 low PLP in cases was due to a decline in albumin, because the association remained after adjustment for albumin level. As 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.

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cent studies have also reported strong inverse correlations between PLP and inflammatory markers in rheumatoid arthritis, a prototypic inflammatory disease.\(^{11}\) 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.

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