B Vitamin Plasma Levels and the Risk of Ischemic Stroke and Transient Ischemic Attack in a German Cohort

Cornelia Weikert, MD, MPH; Jutta Dierkes, PhD; Kurt Hoffmann, PhD; Klaus Berger, MD, MPH; Dagmar Drogan, MPH; Kerstin Klipstein-Grobusch, MSc, PhD; Joachim Spranger, MD; Matthias Möhlig, MD; Claus Luley, MD; Heiner Boeing, MSPH, PhD

Background and Purpose—Data from prospective studies on the associations between B vitamin plasma levels and the risk of stroke are limited. We investigated the individual and combined effects of plasma folate, vitamin B12, and pyridoxal 5-phosphate (PLP) levels on the risk of ischemic stroke and transient ischemic attack (TIA) in a large, prospective German cohort.

Methods—Incident cases of ischemic stroke or TIA were identified among 25 770 participants (age 35 to 65 years) of the European Prospective Investigation into Cancer and Nutrition-Potsdam Study during 6.0±1.5 years of follow-up. The present analysis is based on a case-cohort study comprising 779 subjects free from cardiovascular disease and 188 incident cases of cerebral ischemia (ischemic stroke or TIA). Multivariable Cox proportional-hazard models were applied to evaluate the association between B vitamin levels and risk of cerebral ischemia.

Results—Participants in the lowest tertile of vitamin B12 values were at increased risk of cerebral ischemia compared with subjects in the highest tertile; this was not observed, however, for either folate or PLP. In subgroup analyses, the relative risks were similar in magnitude for stroke and TIA. When various combinations of B vitamin tertile levels were analyzed, only combined low folate and vitamin B12 levels (relative risk, 2.24; 95% CI, 1.10 to 4.54) were significantly related to an increased risk of cerebrovascular ischemia.

Conclusions—Our data suggest that low vitamin B12 plasma levels, particularly in combination with low folate levels, increase the risk of cerebral ischemia. This effect may be mediated at least partly through elevations of homocysteine levels. (Stroke. 2007;38:2912-2918.)

Key Words: cerebrovascular ischemia ■ folate ■ methylene tetrahydrofolate reductase 677 genotype ■ pyridoxal 5-phosphate ■ vitamin B12

Several prospective and intervention studies1 have demonstrated that increased levels of folate and vitamin B12 lower plasma homocysteine levels, an established risk factor for myocardial infarction and stroke.2 These findings formed the basis for large-scale secondary prevention trials3–5 designed to investigate whether the risk of recurrent myocardial infarction, stroke, and other cardiovascular events could be reduced through supplementation with these B vitamins. However, the trials failed to show any significant protective effects of folate and vitamin B12 on cardiovascular risk when the end points (myocardial infarction, stroke, etc) were considered together.

On the other hand, the results of those trials may not be applicable to the long-term development of atherosclerotic changes in the vascular wall preceding the occurrence of clinical end points. For example, the discrepancy between the beneficial cardiovascular effects of estrogen in observational studies in woman compared with secondary prevention trials has been explained in part by differences in atherosclerosis extent.6,7 Thus, the value of B vitamin supplementation in the primary prevention of cardiovascular disease remains elusive, especially when given at an earlier point in the development of atherosclerosis, such as in a primary prevention intervention. Prospective observational studies may provide valuable information on this point by throwing some light on the associations between B vitamins and cardiovascular risk in the primary prevention environment.

Another unresolved issue is that the relevance of B vitamins may differ depending on the type of vascular disease; ie, it may be different for myocardial infarction and...
ischemic stroke. Few prospective studies have investigated the associations between folate or vitamin B12 plasma levels and the risk of stroke and results have been inconsistent.8–11 Moreover, because the relation between vitamin B6 (pyridoxal 5-phosphate [PLP]), another B vitamin involved in homocysteine metabolism, and cerebrovascular ischemia has not yet been the subject of prospective studies, the associations between B vitamin levels and risk of primary cerebrovascular events still remain unclear. Also, there are no prospective data on the combined effects of B vitamin plasma levels on the risk of developing primary cerebrovascular events. We therefore aimed at examining the associations between B vitamin plasma levels and the risk of primary ischemic cerebrovascular events, including ischemic stroke and transient ischemic attack (TIA), within the framework of a large, prospective German cohort study.

Subjects and Methods

Study Population
The European Prospective Investigation into Cancer and Nutrition–Potsdam Study is part of a large-scale Europe-wide prospective cohort study, European Prospective Investigation into Cancer and Nutrition, and includes 27,548 individuals (16,644 women and 10,904 men) mostly between 35 and 65 years old at baseline.12 The participants, who were recruited between 1994 and 1998 from the general population, underwent a baseline examination including standardized blood pressure measurements, measurements of weight and height, self-administered questionnaires on diet and lifestyle, PC-guided interviews, and blood sampling. All gave their written, informed consent, and the ethics committee of the Federal State Brandenburg approved all study procedures. Information about changes in lifestyle and incidents of illness is assessed biennially by self-administered questionnaires.13 Complete filled-in questionnaires were available for 96%, 96%, and 93% of participants after 2, 4, and 6 years, respectively. Only 1.3% of participants were lost to follow-up, and 1.3% were censored before the first follow-up.

After excluding subjects with a history of stroke, myocardial infarction, or TIA, we identified 117 individuals with incidents of TIA and 120 with incidents of stroke (92 ischemic strokes, 24 hemorrhagic, and 4 of undefined etiology) among 25,770 participants during a mean ±SD follow-up of 6.0±1.5 years. Individuals with incident hemorrhagic stroke or stroke of undefined etiology were not considered cases. To analyze the association of biomarkers with the risk of ischemic stroke or TIA, we used a case-cohort design.14,15 This study design can reduce the number of samples to be analyzed for biomarker levels. With a random selection of a subcohort in this type of study design, the results are expected to be generalizable to the entire cohort without the need to measure biomarker levels in the entire cohort.14 For this purpose, a subcohort comprising 821 individuals with sufficient blood samples was drawn from the source population (N = 25,770). Five of the 92 incidents of ischemic stroke and 1 of the 117 incidents of TIA formed part of the subcohort. For the present analyses, we excluded 10 cases of ischemic stroke (1 inside and 9 outside the subcohort), 11 cases of TIA (outside the subcohort), and 36 noncases with missing information for B vitamin levels and/or genotype. Thus, the final case cohort comprised a total of 967 participants, including 188 cases (106 with TIA and 82 with ischemic stroke).

Ascertainment of Stroke and TIA
Every 2 years, participants were asked to complete a mailed follow-up questionnaire that included a section on self-reporting of stroke. In addition, they had to answer a question about cerebral ischemia at the first follow-up and to specify stroke symptoms in the second follow-up questionnaire.16 Subsequent validation of self-reported strokes and TIAs was based on medical records and followed an established protocol that included a standardized form filled in by the treating physician or the study physician.17 We have previously reported on the incidence rate of stroke in this cohort, which roughly resembles the incidence usually observed in the general population of the respective age group in Germany.18,19

Stroke was defined as a focal neurologic deficit with a sudden onset and vascular mechanism lasting ≥24 hours, and a TIA was defined as a neurologic deficit lasting <24 hours. Strokes were classified as ischemic stroke (ICD-10 I63.0-I63.9), intracerebral stroke (ICD-10 I61.0-I61.9), subarachnoidal hemorrhage (ICD-10 I60.0-I60.9), undetermined stroke (ICD-10 I64.0-I64.9), and TIA (ICD-10 G45.0-G45.9) by 2 physicians at the study center.20

Assessment of Risk Factors and Covariates
Lifestyle characteristics, including regular physical exercise and smoking history, were documented at baseline by trained interviewers during a PC-guided interview. Physical activity was defined as the mean time spent on sport activities during summer and winter in hours per week.

Anthropometric data and blood pressure were measured by trained and quality-monitored personnel.21 The body mass index was calculated as body weight divided by height squared (kg/m²). The presence of hypertension was defined, on the basis of mean values of the second and third measurements during the baseline examinations, as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, and/or from the subject’s reports, disease-specific medications, or verification from medical records or the treating physician. The definition of prevalent diabetes was based on the subject’s reports that were checked by a study physician in a telephone interview, disease-specific medication, and/or verification from medical records or the treating physician.

Dietary habits during the preceding year were assessed at baseline by a validated self-administered food frequency questionnaire.22 The food frequency questionnaire included questions on the frequency and portion size of 146 individual food items eaten and drunk during the preceding year, as well as questions on the regular use of vitamin supplements (during the preceding year); this has been described in detail elsewhere.23

Blood Collection and Laboratory Analyses
A total of 30 mL of venous blood was collected from each study participant at the baseline examination at the Potsdam center. The plasma was separated from blood cells according to a standardized protocol, and all samples were frozen at −80°C until the time of analysis.

All plasmas included in this analysis were transferred to the Institute of Clinical Chemistry on dry ice for analyses of folate, cobalamin, PLP, and homocysteine, which were performed by technical assistants blinded to the sample’s casecontrol status. Plasma folate and cobalamin values were measured with commercial test kits (Roche Diagnostics, Mannheim, Germany). The intra-assay coefficient of variation (CV) and interassay CV were 5.0% and 6.0%, and 4.3% and 5.4%, respectively. PLP was determined by high-performance liquid chromatography with fluorescence detection with a commercial test assay (Immundiagnostik GmbH, Bensheim, Germany). Homocysteine was determined by high-performance liquid chromatography with fluorescence detection. The intra-assay CV and interassay CV were 2.4% and 6.8%, and 6.8% and 5.8%, respectively. At the German Institute of Human Nutrition Potsdam, total and HDL cholesterol values were measured with the use of standard methods and reagents from Horiba ABX (Shefford, UK). The intra-assay CV and interassay CV were 0.9% and 1.2%, and 4.7% and 5.2%, respectively.

DNA was isolated from peripheral blood cells and amplified by polymerase chain reaction. The C677T genotype of methylene tetrahydrofolate reductase (MTHFR) was determined by restriction fragment length polymorphism, as previously described by Kluijtmans et al.24

Statistical Analysis
Statistical analysis was performed with the SAS software package, version 9.1 (SAS Institute, Cary, NC). All tests were 2 sided, P < 0.05.
being considered statistically significant. Descriptive statistics of potential confounding variables are given by tertiles of vitamin plasma levels based on the subcohort’s distribution. In view of the skewed distribution of vitamin plasma levels, medians and interquartile ranges are given. Correlations between B vitamins and homocysteine were assessed with Spearman’s rank correlations.

Cox proportional-hazards regression analysis was used for examination of the relation between vitamin levels and risk of cerebral ischemia. Age was the underlying time variable in the counting process, with entry defined as the subject’s age at the time of recruitment and exit defined as the age at diagnosis of cerebral ischemia or censoring. As suggested by Prentice,14 the Cox models were modified to account for the case-cohort design. Relative risks (RRs; hazard rate ratios) of cerebral ischemia were estimated for tertiles of vitamin levels and sex, and all results are therefore given for the 2 sexes together. Table 2 shows RRs of stroke and TIA by tertiles of B vitamin plasma levels. Vitamin B12 plasma levels were inversely associated with the risk of cerebral ischemia in both sex-adjusted and multivariable-adjusted analyses (RR for the lowest versus highest tertile = 1.16 to 2.68), whereas folate and PLP were not associated with these established strata (combinations of vitamins) were estimated in sex-adjusted and multivariable-adjusted Cox models, with the stratum with vitamin levels of all vitamins above the lower tertile being considered statistically significant. Descriptive statistics of potential confounding variables are given by tertiles of vitamin plasma levels based on the subcohort’s distribution. In view of the skewed distribution of vitamin plasma levels, medians and interquartile ranges are given. Correlations between B vitamins and homocysteine were assessed with Spearman’s rank correlations.

Cox proportional-hazards regression analysis was used for examination of the relation between vitamin levels and risk of cerebral ischemia. Age was the underlying time variable in the counting process, with entry defined as the subject’s age at the time of recruitment and exit defined as the age at diagnosis of cerebral ischemia or censoring. As suggested by Prentice,14 the Cox models were modified to account for the case-cohort design. Relative risks (RRs; hazard rate ratios) of cerebral ischemia were estimated for tertiles of vitamin levels and sex, and all results are therefore given for the 2 sexes together. Table 2 shows RRs of stroke and TIA by tertiles of B vitamin plasma levels. Vitamin B12 plasma levels were inversely associated with the risk of cerebral ischemia in both sex-adjusted and multivariable-adjusted analyses (RR for the lowest versus highest tertile = 1.16 to 2.68), whereas folate and PLP were not associated with these established strata (combinations of vitamins) were estimated in sex-adjusted and multivariable-adjusted Cox models, with the stratum with vitamin levels of all vitamins above the lower tertile being the reference category. The association of vitamin status with the risk of cerebral ischemia was calculated after adjustment for age and sex (model 1), additionally for smoking (never smoked; past smoker ≥5 years; past smoker <5 years; current smoker <20 cigarettes; or current smoker ≥20 cigarettes), hypertension, diabetes, total to HDL cholesterol ratio, body mass index, physical activity (<2 or ≥2 h/wk), educational attainment (vocational school or less, technical school, or university), total energy intake (model 2), and further for homocysteine (model 3). To allow an analysis of the effects of specific combinations of B vitamins, such as combined low plasma levels, dichotomized variables of plasma folate, vitamin B12, and PLP were used. Teriles are based on distributions of vitamins in the subcohort. As appropriate, the highest tertile was used as the reference category. The association of vitamin status with the risk of cerebral ischemia was calculated after adjustment for age and sex (model 1), additionally for smoking (never smoked; past smoker ≥5 years; past smoker <5 years; current smoker <20 cigarettes; or current smoker ≥20 cigarettes), hypertension, diabetes, total to HDL cholesterol ratio, body mass index, physical activity (<2 or ≥2 h/wk), educational attainment (vocational school or less, technical school, or university), total energy intake (model 2), and further for homocysteine (model 3). To allow an analysis of the effects of specific combinations of B vitamins, such as combined low plasma levels, dichotomized variables of plasma folate, vitamin B12, and PLP were used. Teriles are based on distributions of vitamins in the subcohort. As appropriate, the highest tertile was used as the reference category. The association of vitamin status with the risk of cerebral ischemia was calculated after adjustment for age and sex (model 1), additionally for smoking (never smoked; past smoker ≥5 years; past smoker <5 years; current smoker <20 cigarettes; or current smoker ≥20 cigarettes), hypertension, diabetes, total to HDL cholesterol ratio, body mass index, physical activity (<2 or ≥2 h/wk), educational attainment (vocational school or less, technical school, or university), total energy intake (model 2), and further for homocysteine (model 3). To allow an analysis of the effects of specific combinations of B vitamins, such as combined low plasma levels, dichotomized variables of plasma folate, vitamin B12, and PLP were used. Teriles are based on distributions of vitamins in the subcohort. As appropriate, the highest tertile was used as the reference category. The association of vitamin status with the risk of cerebral ischemia was calculated after adjustment for age and sex (model 1), additionally for smoking (never smoked; past smoker ≥5 years; past smoker <5 years; current smoker <20 cigarettes; or current smoker ≥20 cigarettes), hypertension, diabetes, total to HDL cholesterol ratio, body mass index, physical activity (<2 or ≥2 h/wk), educational attainment (vocational school or less, technical school, or university), total energy intake (model 2), and further for homocysteine (model 3). To allow an analysis of the effects of specific combinations of B vitamins, such as combined low plasma levels, dichotomized variables of plasma folate, vitamin B12, and PLP were used. Teriles are based on distributions of vitamins in the subcohort. As appropriate, the highest tertile was used as the reference category. The association of vitamin status with the risk of cerebral ischemia was calculated after adjustment for age and sex (model 1), additionally for smoking (never smoked; past smoker ≥5 years; past smoker <5 years; current smoker <20 cigarettes; or current smoker ≥20 cigarettes), hypertension, diabetes, total to HDL cholesterol ratio, body mass index, physical activity (<2 or ≥2 h/wk), educational attainment (vocational school or less, technical school, or university), total energy intake (model 2), and further for homocysteine (model 3). To allow an analysis of the effects of specific combinations of B vitamins, such as combined low plasma levels, dichotomized variables of plasma folate, vitamin B12, and PLP were used. Teriles are based on distributions of vitamins in the subcohort. As appropriate, the highest tertile was used as the reference category. The association of vitamin status with the risk of cerebral ischemia was calculated after adjustment for age and sex (model 1), additionally for smoking (never smoked; past smoker ≥5 years; past smoker <5 years; current smoker <20 cigarettes; or current smoker ≥20 cigarettes), hypertension, diabetes, total to HDL cholesterol ratio, body mass index, physical activity (<2 or ≥2 h/wk), educational attainment (vocational school or less, technical school, or university), total energy intake (model 2), and further for homocysteine (model 3). To allow an analysis of the effects of specific combinations of B vitamins, such as combined low plasma levels, dichotomized variables of plasma folate, vitamin B12, and PLP were used. Teriles are based on distributions of vitamins in the subcohort. As appropriate, the highest tertile was used as the reference category. The association of vitamin status with the risk of cerebral ischemia was calculated after adjustment for age and sex (model 1), additionally for smoking (never smoked; past smoker ≥5 years; past smoker <5 years; current smoker <20 cigarettes; or current smoker ≥20 cigarettes), hypertension, diabetes, total to HDL cholesterol ratio, body mass index, physical activity (<2 or ≥2 h/wk), educational attainment (vocational school or less, technical school, or university), total energy intake (model 2), and further for homocysteine (model 3). To allow an analysis of the effects of specific combinations of B vitamins, such as combined low plasma levels, dichotomized variables of plasma folate, vitamin B12, and PLP were used. Teriles are based on distributions of vitamins in the subcohort. As appropriate, the highest tertile was used as the reference category.
levels alone, those with a combination of low folate and low B12 levels, and those with low levels of all 3 B vitamins were at a significantly increased risk of cerebral ischemia compared with their counterparts for whom all B vitamin levels were in the second or third tertile. Low folate and PLP levels, alone or in combination, were not associated with the outcome.

Adjustment for hypertension, smoking, diabetes, alcohol consumption, body mass index, education, physical activity, and the total to HDL cholesterol ratio did not significantly alter the association between the risk of cerebral ischemia and combined low folate and vitamin B12 (RR = 2.24; 95% CI, 1.10 to 4.54), whereas isolated low vitamin B12 levels and combined low levels of all B vitamins investigated were no longer significantly associated with risk. After further adjustment for homocysteine, all associations became considerably weaker and were no longer significant, indicating that the effect of low vitamin B12 or folate levels was linked to high homocysteine levels.

As expected, participants with low vitamin B plasma levels were less likely to use vitamin supplements. The percentages of supplement users among the participants with low vitamin

<table>
<thead>
<tr>
<th>Table 1. Continued</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tertiles of Vitamin B12</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>40.6</td>
</tr>
<tr>
<td>50.0±8.6</td>
</tr>
<tr>
<td>19.4</td>
</tr>
<tr>
<td>25.8±3.5</td>
</tr>
<tr>
<td>8 (3–17)</td>
</tr>
<tr>
<td>52.2</td>
</tr>
<tr>
<td>2.1</td>
</tr>
<tr>
<td>9.3</td>
</tr>
<tr>
<td>9.2 (7.6–11.6)</td>
</tr>
<tr>
<td>18.8 (15.0–24.5)</td>
</tr>
<tr>
<td>30.7 (21.4–43.7)</td>
</tr>
<tr>
<td>47.8</td>
</tr>
<tr>
<td>43.9</td>
</tr>
<tr>
<td>8.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2.</th>
<th><strong>RRs of Cerebral Ischemia According to Tertiles of Plasma B Vitamins</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertiles of Individual B Vitamins</td>
<td>Cases of Stroke/TIA</td>
</tr>
<tr>
<td>Folate</td>
<td></td>
</tr>
<tr>
<td>3 (Reference)</td>
<td>34/34</td>
</tr>
<tr>
<td>2</td>
<td>19/33</td>
</tr>
<tr>
<td>1</td>
<td>29/39</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td></td>
</tr>
<tr>
<td>3 (Reference)</td>
<td>24/32</td>
</tr>
<tr>
<td>2</td>
<td>20/37</td>
</tr>
<tr>
<td>1</td>
<td>38/37</td>
</tr>
<tr>
<td>PLP</td>
<td></td>
</tr>
<tr>
<td>3 (Reference)</td>
<td>28/29</td>
</tr>
<tr>
<td>2</td>
<td>20/40</td>
</tr>
<tr>
<td>1</td>
<td>34/37</td>
</tr>
</tbody>
</table>

All models were derived from a Cox proportional-hazards regression with age as the underlying time variable, stratified by age at baseline.

*Model 2 includes age, sex, smoking status, diabetes, hypertension, total to HDL cholesterol ratio, education, alcohol intake, physical activity, body mass index, and total energy intake.

†Model 3 is model 2 plus homocysteine.
Our findings are in contrast to previous studies, in which it was found that folate and PLP rather than vitamin B12 were associated with risk of cerebrovascular events. Inverse associations between plasma folate and risk of cerebral ischemia have been observed in some previous studies. Moreover, the reduction in stroke mortality after widespread vitamin B12 alone, combined low vitamin B12 and PLP levels, and combined low levels of all 3 B vitamins. The risk increase associated with low vitamin B12 and folate became weaker nonsignificant after adjustment for homocysteine plasma levels, suggesting that the effect of these B vitamins may be mediated in part by their influence on homocysteine metabolism.

Our findings are in contrast to previous studies, in which it was found that folate and PLP rather than vitamin B12 were associated with risk of cerebrovascular events. Inverse associations between plasma folate and risk of cerebral ischemia have been observed in some previous studies. Moreover, the reduction in stroke mortality after widespread folate acid fortification of grain products in the United States and Canada supports the putative effect of folic acid on the development of cerebrovascular diseases. On the other hand, our finding of a null association between folate and cerebral ischemia is in line with 2 recently published cohort studies. In this context, it may be important that in our study population, folate plasma levels were comparatively high. As a consequence, the range of folate levels in this study may only partly fit in with biologically relevant serum values.

According to a recent study by Quinlivan et al., vitamin B12 levels may determine homocysteine levels and cerebrovascular risk, because the general folate status has been improved by the fortification of grain products in the United States and Canada. A subgroup analysis of the VISP trial excluding participants with very low or very high vitamin B12 levels at baseline showed a benefit of high-dose B vitamin supplementation compared with a low dose for the combined end point of ischemic stroke, coronary disease, and death. These findings suggest that there may be a relevant subgroup that is more likely to benefit from vitamin therapy. Moreover, this study showed that patients with a high baseline vitamin B12 level who received high-dose supplementation had the best overall outcome. Concordant with those results, our findings support the concept of a protective role for vitamin B12 in the development of stroke. On the other hand, we cannot exclude the possibility that the observed associations between B vitamin levels and stroke risk may be attributable in part to residual confounding by other, as-yet-unidentified factors. Data from case-control studies also suggest a protective effect of high PLP levels on stroke. However, we did not find an independent association between PLP and our end points.

To our knowledge, this is the first prospective study to investigate the combined effects of B vitamin levels on cerebrovascular risk. We observed an increased risk in subjects with low B12 levels, particularly in those with combined low folate and low vitamin B12 levels. The associations between folate and PLP plasma levels and risk appeared to be dependent on vitamin B12 levels, assigning to B12 a crucial role in our study. Nevertheless, the protective effect of B vitamins may depend on sufficient plasma levels of all 3 vitamins, even if vitamin B12 is particularly important. Previous studies did not consider the combined effects of
B vitamins, which may explain, at least in part, why some of those investigations failed to detect any associations between B vitamins and stroke.

Remarkably, subjects with low B vitamin levels were taking vitamin supplements less frequently than were other participants in the study. However, the use of vitamins was not independently associated with the risk of cerebral ischemia. In this context, it is worth noting that in 1 large intervention trial for secondary prevention, the HOPE 2 study, patients assigned to active treatment with folic acid, vitamin B12, and vitamin B6 were at a lower risk of recurrent stroke (RR = 0.75, 95% CI, 0.59 to 0.97) compared with the placebo group, although the authors themselves regarded this as a chance finding. Moreover, a nonsignificant reduction in risk of recurrent stroke (odds ratio = 0.83; 95% CI, 0.47 to 1.47) was observed in the intervention group receiving 3 B vitamins in the NORVIT study. Further analyses based on the NORVIT data revealed that supplementation with folic acid plus vitamin B12 or vitamin B6 had a slightly beneficial effect on most clinical outcomes. Moreover, those studies suggested an interaction between folic acid and vitamin B6 that led to significantly worse outcomes, whereas there was no evidence that treatment with folic acid plus vitamin B12 or with vitamin B12 alone was harmful. The findings of our study suggest a detrimental effect of low B vitamin levels, particularly of low vitamin B12 levels, on the development of cerebrovascular disease.

Some limitations of the present study should be mentioned. In the first place, because of the relatively short follow-up and the age distribution of the study population, the number of cases was rather limited, thus reducing the power of our stratified analyses according to tertiles of B vitamin levels. Moreover, insufficient statistical power hampered further stratified analyses, eg, those relating to the MTHFR polymorphism. Second, our results were based on single measurements of plasma folate, vitamin B12, and PLP. Thus, we cannot entirely exclude the possibility that measurement errors biased our results toward the null value, although we believe that the potential for such bias, if any, is low. Among the strengths of this study are its prospective study design, the blinded measurements of biomarkers, and the comprehensive data on study participants that allowed for adjustment for other risk factors. All cases of stroke and TIA were validated by medical records, treating physicians, or death certificates, providing high accuracy in identifying incident cases.

Taken together, our findings support further studies into the role of the B vitamins in the primary prevention of stroke. In particular, it is still unclear whether there are certain subgroups of people who may profit most from B vitamin supplementation. In this context, genetic factors may greatly influence an individual’s response to B vitamin supplementation. However, we did not observe any association between the MTHFR C677T genotype and risk of cerebral ischemia in our study, which is in line with other studies from central and northern Europe. In conclusion, low vitamin B12, particularly in combination with low folate, may constitute a risk-increasing factor for the development of TIA and stroke. For the putative protective effect of B vitamins, certain thresholds of folate, vitamin B12, and PLP seem to be crucial.

Further studies into the role of B vitamins and B vitamin supplementation in the primary prevention of cerebrovascular ischemia appear justified.

Acknowledgments

We are indebted to Manuela Bergmann and Wolfgang Fleischhauer for case ascertainment, Ellen Kohlwald for data management, and Elke Hinze for the laboratory measurements.

Source of Funding

The present study was supported by the Federal Ministry of Education and Research (BMBF, research grant No. 0312750B).

Disclosures

None.

References

B Vitamin Plasma Levels and the Risk of Ischemic Stroke and Transient Ischemic Attack in a German Cohort

Cornelia Weikert, Jutta Dierkes, Kurt Hoffmann, Klaus Berger, Dagmar Drogan, Kerstin Klipstein-Grobusch, Joachim Spranger, Matthias Möhlig, Claus Luley and Heiner Boeing

Stroke. 2007;38:2912-2918; originally published online September 20, 2007;
doi: 10.1161/STROKEAHA.107.486068

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/38/11/2912