B Vitamins and Magnetic Resonance Imaging–Detected Ischemic Brain Lesions in Patients With Recent Transient Ischemic Attack or Stroke

The VITAmins TO Prevent Stroke (VITATOPS) MRI-Substudy

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Background and Purpose—Elevated concentrations of homocysteine are associated with cerebral small vessel disease (CSVD). B-vitamin supplementation with folate and vitamins B₁₂ and B₆ reduces homocysteine concentrations. In a substudy of the VITAmins TO Prevent Stroke (VITATOPS) trial, we assessed the hypothesis that the addition of once-daily supplements of B vitamins would reduce the progression of CSVD-related brain lesions.

Methods—A total of 359 patients with recent stroke or transient ischemic attack, who were randomly allocated to double-blind treatment with placebo or B vitamins, underwent brain MRI at randomization and after 2 years of B-vitamin supplementation. MR images were analyzed blinded to treatment allocation. Outcomes related to the prespecified hypothesis were progression of white matter hyperintensities and incident lacunes. We also explored the effect of B-vitamin supplementation on the incidence of other ischemic abnormalities.

Results—After 2 years of treatment with B vitamins or placebo, there was no significant difference in white matter hyperintensities volume change (0.08 vs 0.13 cm³; P=0.419) and incidence of lacunes (8.0% vs 5.9%, P=0.434; odds ratio=1.38). In a subanalysis of patients with MRI evidence of severe CSVD at baseline, B-vitamin supplementation was associated with a significant reduction in white matter hyperintensities volume change (0.3 vs 1.7 cm³; P=0.039).

Conclusions—Daily B-vitamin supplementation for 2 years did not significantly reduce the progression of brain lesions resulting from presumed CSVD in all patients with recent stroke or transient ischemic attack but may do so in the subgroup of patients with recent stroke or transient ischemic attack and severe CSVD.


Key Words: cerebral small vessel diseases ■ folic acid ■ homocysteine ■ randomized controlled trial ■ stroke ■ vitamin B deficiency

Elevated blood concentrations of total homocysteine have been associated with cerebral small vessel disease (CSVD) as defined by clinical lacunar syndromes or brain magnetic resonance imaging (MRI) evidence of ischemic lesions in the vascular territories of the deep perforating small arteries.1,3 Homocysteine can be lowered by B-vitamin supplementation with folate alone or in combination with vitamins B₁₂ and B₆.4,5

In a substudy of the VITAmins TO Prevent Stroke (VITATOPS) trial, we assessed the prespecified hypothesis...
that addition of once-daily supplements of B vitamins would reduce the progression of CSVD on brain MRI.

Methods

Setting and Patients

The rationale and design of the VITATOPS trial have been published previously.6 Briefly, VITATOPS was a prospective, randomized, double-blind, placebo-controlled clinical trial. Patients were eligible if they had had a stroke (ischemic or hemorrhagic) or a transient ischemic attack in the past 7 months.

The brain MRI substudy of the VITATOPS trial was undertaken in five centers (Department of Neurology, Medical University of Graz, Graz, Austria; Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong Special Administrative Region, China; Neurology Department, Royal Perth Hospital, Perth, WA; Universidade Federal do Rio de Janeiro/ D’Or Institute for Research and Education [IDOR], Rio de Janeiro, Brazil; Singapore General Hospital, Singapore) and included patients who underwent a brain MRI scan according to a standardized protocol at the baseline evaluation.

Figure 1 shows the flow of patients in the study. A total of 471 patients met the study entry criteria and underwent a baseline MRI scan. After 2 years, 383 (81.3%) underwent a follow-up MRI scan. The brain MRI substudy of the VITATOPS trial was undertaken in five centers (Department of Neurology, Medical University of Graz, Graz, Austria; Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong Special Administrative Region, China; Neurology Department, Royal Perth Hospital, Perth, WA; Universidade Federal do Rio de Janeiro/ D’Or Institute for Research and Education [IDOR], Rio de Janeiro, Brazil; Singapore General Hospital, Singapore) and included patients who underwent a brain MRI scan according to a standardized protocol at the baseline evaluation.

Figure 1 shows the flow of patients in the study. A total of 471 patients met the study entry criteria and underwent a baseline MRI brain scan. After 2 years, 383 (81.3%) underwent a follow-up MRI brain scan; the reasons for 88 patients to not have a follow-up MRI brain scan at baseline and follow-up. Their mean age was 64.3 ± 12.7 years, and 228 (63.7%) participants were men. Ninety-one subjects (20.5%) had transient ischemic attacks, 345 (77.5%) ischemic and 9 (2.0%) hemorrhagic strokes.

Standardized Brain MRI Scanning

Procedure

The participating sites were free to use the set of sequences that was regularly used at their center. All scans were obtained on 1.5 Tesla scanners. Axial T2 or FLAIR images and a diffusion-weighted imaging sequence were mandatory. T1 and T1*-weighted sequences were adequate quality, leaving 359 participants with acceptable quality MRI scans. Axial T2 or FLAIR images and a diffusion-weighted imaging sequence were mandatory. T1 and T1*-weighted sequences were recommended but optional.

Analysis

MR images were analyzed centrally at the Department of Neurology, Medical University of Graz, Austria by three trained readers (M.C., A.G., and N.H.). The readers were masked to any information about the demographic and clinical features of the patient or treatment allocation and recorded all imaging findings according to a prespecified protocol. Image quality was rated as poor, sufficient, or good.

The readers recorded all lesions on the baseline scans and evaluated lesion change by comparison with the follow-up scans in a side-by-side fashion with knowledge of the date of the examination. Weekly reading sessions between the readers and the principal investigator (R.S.) served to reach a consensus on lesion change from baseline in case of uncertainties.

White matter hyperintensities (WMH) progression and incident lacunes were the primary prespecified study outcomes. White matter hyperintensities were defined as lesions of high signal intensity on T2-weighted and FLAIR images in the absence of evidence for complete tissue destruction. They were rated at baseline according to the Fazekas scale7 as deep WMH (0=absent; 1=punctuate; 2=early confluent; 3=confluent) and periventricular WMH (0=absent; 1=pencil-thin lining; 2=halo of ≥5 mm thickness). This rating has been shown to have high intra- and inter-rater reliability.7 WMH progression was assessed semiautomatically using volumetric measurement. The volume of WMH at baseline and the longitudinal volume change were measured as previously described.10 In brief, lesion load measurements were done on FLAIR images on an LINUX workstation by a trained operator using DISPImage.11 All lesions were outlined on the computer image, and the lesion areas were then provided by the DISPImage program. The total lesion volume (cm³) was calculated by multiplying the total lesion area by slice thickness. Details on methodology and the reproducibility of the volumetric assessments of white matter lesions have been described.10 WMH volume includes supratentorial and infratentorial WMH abnormalities. Lacunes, cortical lesions, and basal ganglia lesions were excluded from the volumetric analysis.

Lacunes were defined as focal lesions of cerebrospinal fluid signal intensity that involved the basal ganglia, internal capsule, thalamus, white matter, or brain stems, not exceeding a diameter of 15 mm. Incident lacunes were consistent lesions not present on the baseline scan.

If study participants had a deep WMH score ≥2 and lacunes (acute or old) at baseline we considered them as having severe CSVD. This definition intended to select a subgroup of patients at high risk of progression of CSVD.10

All scans were also evaluated for thromboembolic infarcts and intracerebral hemorrhages. Thromboembolic infarcts were classified by type (territorial and watershed), acuteness (by diffusion-weighted imaging), and number. Cerebral hemorrhages and microbleeds were recorded in number and location, but their number was too low to allow for meaningful statistical analysis. Data on hemorrhagic lesions are therefore not displayed.

Intervention

Patients were randomly assigned to receive either B vitamins (2 mg folic acid, 25 mg vitamin B6, and 0.5 mg vitamin B12) or placebo. Patients, clinicians, trial coordinators, and outcome investigators were blinded to treatment allocation.

Statistical Analysis

Baseline characteristics were tabulated according to the assigned treatment groups. Categorical variables were expressed as proportions and compared by χ² test, continuous variables were shown as means (SD) or median (range) and compared by t test or Wilcoxon’s rank test depending on presence or absence of normal distribution, respectively.

The prespecified outcome measures of the study were progression of WMH and incident lacunes. We also assessed the differences between actively or placebo-treated patients for incidence of thromboembolic infarcts. Post hoc analyses were done in the subsample of...
those 100 study participants with severe CSVD (deep WMH score >2 and lacunes) at baseline because this group of patients already manifested the disease that the intervention was targeted to and had higher homocysteine levels at baseline than patients without severe CSVD, thus increasing the likelihood of identifying an effect of lowering homocysteine with B vitamins on the progression of CSVD if one truly existed.

To assess the possible effect of age on treatment response we performed a linear regression analysis with log-transformed change in WMH volume as the dependent variable and age, treatment status, and the interaction term age × treatment status as the independent variables.

An identical analysis was also done including lacunar stroke/nonlacunar stroke, treatment status, and the interaction term lacunar stroke/nonlacunar stroke × treatment status.

All analyses were performed using STATA statistical software (release 9; College Station, TX).

## Results

A total of 174 subjects were randomly assigned to receive vitamin supplementation, and 185 were on placebo. There were no significant differences in demographic and clinical characteristics between the two groups (Table 1).

Baseline MRI findings were also similar (Table in the online-only Data Supplement).

As can be seen from Table 2, after 2 years (mean 24.6 ± 2.7 months) of treatment with B vitamins or placebo progression of WMH and frequency of incident lacunes were not significantly different between the investigational subsets. The distribution of incident thromboembolic infarcts was also similar. Table 3 shows the results of the subanalysis of those 100 patients with severe CSVD at baseline. In this subset of patients B-vitamin supplementation was associated with a significant reduction in WMH volume change. No significant differences were seen for the incidence of lacunes or other brain lesions. Baseline characteristics and homocysteine concentrations between the severe CSVD subgroups according to treatment assignment were comparable (data not shown). As expected, the severe CSVD group was older (69.6 vs 62.2 years; *P* < 0.001) and had higher systolic pressure (147.6 vs 139.7 mmHg; *P* < 0.001) and higher homocysteine levels at baseline (15.9 vs 13.5 μmol/L; *P* = 0.004) than their counterpart without severe CSVD. Diastolic blood pressure was also higher, but the between-group difference did not reach statistical significance (84.8 vs 82.3 mmHg; *P* = 0.062). There existed no significant interaction between age and treatment status on WMH volume change. This was seen in the overall sample (β = 0.0002, *P* = 0.999) and in the group with severe CSVD (β = −0.011; *P* = 0.976). The interaction term lacunar stroke/nonlacunar stroke × treatment was also neither significant in the total cohort (β = −0.017; *P* = 0.894) nor in the CSVD subgroup (β = −0.11; *P* = 0.809).

## Discussion

In our study daily administration of folic acid, vitamin B_{12}, and vitamin B_{6} for 2 years did not significantly reduce the progression of brain lesions resulting from presumed cerebral small vessel disease in all patients with recent stroke or transient ischemic attack. However, a post hoc subgroup analysis in patients with MRI evidence of severe cerebral small vessel disease at baseline, who we felt would be those who would be most likely to show a response to B-vitamin treatment over 2 years, raises the hypothesis that B-vitamin supplementation may slow the progression of WMH. Given the neutral outcome of the whole VITATOPS MRI substudy but also that of the whole VITATOPS trial, the results of our post hoc analysis in patients with severe CSVD have to be interpreted with caution.

Previous cross-sectional studies reported a direct association between homocysteine concentrations and WMH volume. Although the VITATOPS trial showed that daily B vitamin supplementation after a recent stroke or transient ischemic attack was not significantly more effective than placebo in reducing the incidence of major vascular events, a subgroup analysis in etiological stroke subsets suggested that this...
therapeutic intervention reduces the risk of recurrent vascular events in subjects with lacunar stroke.\textsuperscript{14} We did not find significant effects on incident lacunes either in the whole cohort or in the subset of participants with severe CSVD at baseline. Notably, the number of subjects with incident lacunes was small.

In line with previous literature our patients with severe CSVD also had higher homocysteine levels than the total cohort including strokes of all etiologies. We were unable to determine whether homocysteine lowering in the actively treated group was indeed responsible for the slowing of WMH progression because longitudinal homocysteine measurements were not available. Nonetheless, it is known that vitamin supplementation in the entire VITATOPS cohort decreased the homocysteine concentration by approximately 4 µmol/L in the treated group as compared with placebo.\textsuperscript{15}

There are several mechanisms by which homocysteine lowering may slow white matter lesion progression.\textsuperscript{15} First, hyperhomocysteinemia-related endothelial dysfunction may be reduced.\textsuperscript{1} Second, hypomethylation, which affects myelin integrity, was found to be linked to homocysteine levels and lowering of the homocysteine concentration may thus exert favorable effects with respect to both de- and remyelination processes.\textsuperscript{16} Third, homocysteine lowering may lead to attenuation of its direct neurotoxic effects of via its action as an N-methyl-D-aspartate agonist.\textsuperscript{17} In this context it is important to note that a recent study reported reduction of leukoaraisis in a rat model when the animals were treated with an N-methyl-D-aspartate antagonist.\textsuperscript{18}

Although a selective effect of homocysteine lowering on white matter lesion progression as described in our study is plausible, we cannot exclude with certainty that we are dealing with a false positive result because of the relatively small sample size of subjects with severe CSVD-related MRI abnormalities.

With respect to the generalizability of our results it is important to emphasize that with 64.3 ± 12.7 years the mean age of our VITATOPS substudy sample was similar to that of the entire VITATOPS cohort (62.6 ± 12.5 years) and other clinical trials in stroke patients with long-term follow-up.\textsuperscript{19} Yet, it is lower than can be expected in the general stroke population.\textsuperscript{20} Age was reported as a predictor of WMH progression in previous studies,\textsuperscript{2} and the relatively young age of our study participants might thus have led to a change in WMH volume smaller than that seen in general stroke cohorts. Conceivably, the effect size of vitamin supplementation might be age-dependent. Yet, this is not corroborated by our results, because the interaction term age × treatment status was not significant. There also existed no significant interaction between lacunar stroke and treatment effect on WMH progression.

Noteworthy, this is the first placebo-controlled interventional MRI study on the effects of daily administration of folic acid, vitamin B\textsubscript{6}, and vitamin B\textsubscript{12} in stroke patients. There has been only one other clinical trial using such intervention in healthy siblings of patients with premature atherosclerotic disease. The authors\textsuperscript{22} indicated a favorable effect of homocysteine lowering on cerebrovascular atherosclerosis, and in line with our data they also found less white matter damage.

Our finding may have important clinical implications because white matter abnormalities have been reported to predict disability and mortality in elderly people.\textsuperscript{23,24} Larger trials in patients with MRI evidence for severe CSVD are required to confirm our results. If indeed vitamins slow progression of WMH, the effect will be seen most easily in patients with the most severe WMH where the control group’s WMH are progressing most rapidly.

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### Disclosures

Dr Schmidt has received payments for giving lectures from Pfizer, Merz Austria, Novartis, and Lundbeck. Dr Chen has received payments for serving as national coordinator of the PERFORM (Servier) trial, on the data monitoring committee of the DU176 B-C-J226 (Dai-Ichi) trial, as adviser to the ImpACT-24 (Brainsgate) trial, and for being part of a working group on stroke and lipid management in ASIA (Pfizer), and has received travel and accommodation expenses from Molec to attend the European Stroke Congress. The other authors report no conflicts.

### References


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**SUPPLEMENTAL MATERIAL**

Supplemental table: MRI baseline characteristics of patients undergoing repeated MRI scanning (N=359).

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*: Chi-square tests for categorical variables, Wilcoxon’s rank sum tests for continuous variables.

†: number of patients included in the analysis

WMH: white matter hyperintensities, MBs: microbleeds