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

Margherita Cavalieri, MD; Reinhold Schmidt, MD; Christopher Chen, MD; Vincent Mok, MD; Gabriel R. de Freitas; Swithin Song, MBBS; Qilong Yi, MSc, PhD; Stefan Ropele, PhD; Anja Grazer, MD; Nina Homayoon, MD; Christian Enzinger, MD; Katherine Loh; Ka Sing Lawrence Wong, MD; Adrian Wong, PhD; Yunyun Xiong, MD; PhD; Hui Meng Chang, MD; Meng Cheong Wong, MBBS; Franz Fazekas, MD; John W. Eikelboom, MBBS; Graeme J. Hankey, MD; on behalf of the VITATOPS Trial Study Group

Background and Purpose—Elevated concentrations of homocysteine are associated with cerebral small vessel disease (CSVD). B-vitamin supplementation with folate and vitamins B_{12} and B_6 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 cm^3; 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 cm^3; 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.

Clinical Trial Registration—http://vitatops.highway1.com.au/. Unique identifier: NCT00097669 and ISRCTN74743444. **(*Stroke*. 2012;43:3266-3270.)**

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 B_{12} and B_6.[4,5]

In a substudy of the VITAmins TO Prevent Stroke (VITATOPS) trial, we assessed the prespecified hypothesis

Received May 25, 2012; final revision received August 30, 2012; Accepted September 13, 2012.

From the Department of Neurology, Medical University of Graz, Austria (M.C., R.S., S.R., A.G., N.H., C.E., F.F.); the Department of Pharmacology, National University of Singapore, Singapore (C.C., M.C.W.); the Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong Special Administrative Region, China (V.M., K.S.L.W., A.W., Y.X.); Universidade Federal do Rio de Janeiro/ D'Or Institute for Research and Education (IDOR), Rio de Janeiro, Brazil (G.R.d.F.); the Department of Radiology, Royal Perth Hospital, Perth, Western Australia (S.S.); Public Health and Preventive Medicine. University of Ottawa, Ottawa, Ontario, Canada (Q.Y.); Neurology Department, Royal Perth Hospital, Perth, Western Australia (K.L.); the Department of Psychological Studies, The Hong Kong Institute of Education, Hong Kong Special Administrative Region, China (A.W.); the Department of Neurology, Jingling Hospital, Nanjing University school of Medicine, Nanjing, China (Y.X.); the Department of Neurology, National Neuroscience Institute, Singapore (H.M.C.); the Department of Medicine, McMaster University, Hamilton, Canada (J.W.E.).

Natan M Bornstein, MD, was the guest editor for this article.

The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.112.665703/-/DC1.

Correspondence to Reinhold Schmidt, Medical University of Graz, Department of Neurology, Division of Neurogeriatrics, Auenbruggerplatz 22, 8036 Graz, Austria. E-mail reinhold.schmidt@medunigraz.at

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.[6,7] 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 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 are displayed in Figure 1. Among the 383 patients with a follow-up MRI scan, 24 MRI investigations were excluded because of inadequate quality, leaving 359 participants with acceptable quality MRI brain scans 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 T_2 or FLAIR images and a diffusion-weighted imaging sequence were mandatory. T_1 and T_2*-weighted sequences were recommended but optional.

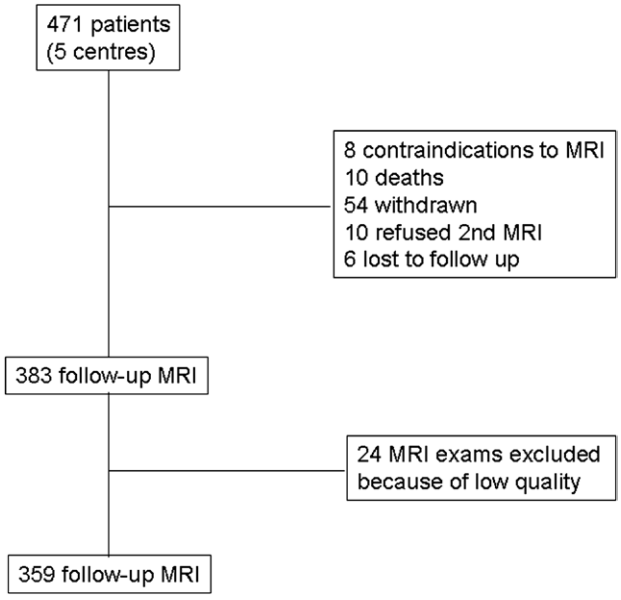

Figure 1. Flowchart of participants in the VITATOPS MRI substudy.

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 T_2-weighted and FLAIR images in the absence of evidence for complete tissue destruction. They were rated at baseline according to the Fazekas scale[8] as deep WMH (0=absent; 1=punctuate; 2=early confluent; 3=confluent) and periventricular WMH (0=absent; 1=pencil-thin lining; 2=halo of ≥5mm thickness). This rating has been shown to have high intra- and inter-rater reliability.[9] 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 (cm^3) 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 B_6, and 0.5 mg vitamin B_{12}) 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 χ^2 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 MRI evidence of 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 mm Hg; $P<0.001$) 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 mm Hg; $P=0.062$). There existed no significant interaction between age and treatment status on WMH volume change. This was seen in the overall sample (beta=0.0002, $P=0.999$) and in the group with severe CSVD (beta=–0.011; $P=0.976$). The interaction term lacunar stroke/nonlacunar stroke × treatment was also neither significant in the total cohort (beta=–0.017; $P=0.947$) nor in the CSVD subgroup (beta=–0.11; $P=0.809$).

Table 1. Demographics and Baseline Characteristics of Patients Undergoing Repeated MRI Scanning (n=359)

	B Vitamins (n=174)		Placebo (n=185)		
	Total†	Value	Total†	Value	*P* Value*
Age, y (mean±SD)	174	64.8 (12.4)	185	63.8 (13.1)	0.513
Men, n (%)	173	119 (64.3)	185	109 (63.0)	0.795
Smoking, n (%)	173	78 (45.1)	185	96 (51.9)	0.198
Systolic blood pressure, mm Hg (mean±SD)	168	140.1 (20.1)	177	143.6 (20.0)	0.102
Diastolic blood pressure, mm Hg (mean±SD)	168	82.2 (12.1)	176	83.6 (10.7)	0.257
Medical history					
Stroke, n (%)	174	31 (17.8)	185	38 (20.5)	0.513
Myocardial infarction, n (%)	174	8 (4.6)	185	4 (2.1)	0.203
Peripheral artery disease, n (%)	174	4 (2.3)	185	4 (2.1)	0.930
Hypertension, n (%)	174	119 (68.4)	185	134 (72.4)	0.401
Hypercholesterolemia, n (%)	161	83 (51.5)	160	75 (46.8)	0.402
Diabetes mellitus, n (%)	174	39 (22.4)	185	50 (27.0)	0.312
Atrial fibrillation, n (%)	174	9 (5.2)	185	14 (7.5)	0.354
Ischemic heart disease, n (%)	174	32 (18.4)	185	26 (14.0)	0.265

*χ^2 tests for categorical variables, *t* tests for continuous variables.
†Number of patients included in the analysis.

Discussion

In our study daily administration of folic acid, vitamin B_6, and vitamin B_{12} 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.[3,12,13] 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

Table 2. MRI Changes in Relation to 2-Year Treatment With B Vitamins or Placebo in the Total Cohort (n=359)

	B Vitamins (n=174)		Placebo (n=185)		
	Total†	Value	Total†	Value	*P* Value*
WMH volume change, cm^3, median (range)	154	0.08 (0–18.9)	163	0.13 (0–13.6)	0.419
Incident lacunes, n (%)	174	14 (8.0)	185	11 (5.9)	0.434
Incident infarcts, n (%)	174	11 (6.3)	185	6 (3.2)	0.286
Type of infarct, n (%)					
Territorial	174	11 (6.3)	185	5 (2.7)	0.096
Watershed	174	—	185	1 (0.5)	1.000

*χ^2 tests for categorical variables, and Wilcoxon's rank sum tests for continuous variables.
†Number of patients included in the analysis.
WMH indicates white matter hyperintensities.

Table 3. MRI Changes in Relation to 2-Year Treatment With B Vitamins or Placebo in a Subset of 100 Patients With Severe SVD (Deep WMH Score ≥2 AND Lacunes)

	B vitamins (n=54)		Placebo (n=46)		
	Total†	Value	Total†	Value	*P* Value*
WMH volume change, cm^3, median (range)	50	0.3 (0–13.6)	44	1.7 (0–13.6)	0.039
Incident lacunes, n (%)	54	9 (16.6)	46	5 (10.8)	0.405
Incident infarcts, n (%)	54	6 (11.1)	46	0	1.000
Type of infarct, n (%)					
Territorial	54	6 (11.1)	46	—	1.000
Watershed	54	—	46	—	—

*χ^2 tests for categorical variables, and Wilcoxon's rank sum tests for continuous variables.

†Number of patients included in the analysis.

WMH indicates white matter hyperintensities.

therapeutic intervention reduces the risk of recurrent vascular events in subjects with lacunar stroke.[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 WML 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.[15]

There are several mechanisms by which homocysteine lowering may slow white matter lesion progression.[15] First, hyperhomocysteinemia-related endothelial dysfunction may be reduced.[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.[16] Third, homocysteine lowering may lead to attenuation of its direct neurotoxic effects of via its action as an *N*-methyl-D-aspartate agonist.[17] In this context it is important to note that a recent study reported reduction of leukoaraiosis in a rat model when the animals were treated with an *N*-methyl-D-aspartate antagonist.[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.[19] Yet, it is lower than can be expected in the general stroke population.[20] Age was reported as a predictor of WMH progression in previous studies,[21] 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_6, and vitamin B_{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[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.[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.

Sources of Funding

The VITATOPS trial was funded by grants from the Australia National Health and Medical Research Council (project grants 110267, 403913, and 572632; program grants 251525 and 454417), the Singapore Biomedical Research Council, the Singapore National Medical Research Council, the Australia National Heart Foundation (grants G 99P 0405, G 02P 0753, G 04P 1611), the Royal Perth Hospital Medical research Foundation, and the Health Department of Western Australia, 2005/2006 Research Grant Council CUHK 4317/04M.

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 Moleac to attend the European Stroke Congress. The other authors report no conflicts.

References

1. Fassbender K, Mielke O, Bertsch T, Nafe B, Fröschen S, Hennerici M. Homocysteine in cerebral macroangiography and microangiopathy. *Lancet*. 1999;353:1586–1587.
2. Eikelboom JW, Hankey GJ, Anand SS, Lofthouse E, Staples N, Baker RI. Association between high homocyst(e)ine and ischemic stroke due to large- and small-artery disease but not other etiologic subtypes of ischemic stroke. *Stroke*. 2000;31:1069–1075.
3. Hassan A, Hunt BJ, O'Sullivan M, Bell R, D'Souza R, Jeffery S, Bamford JM, Markus HS. Homocysteine is a risk factor for cerebral small vessel disease, acting via endothelial dysfunction. *Brain*. 2004;127(pt 1):212–219.
4. Seshadri S, Wolf PA. Homocysteine and the brain: vascular risk factor or neurotoxin? *Lancet Neurol*. 2003;2:11.

5. Homocysteine Lowering Trialists' Collaboration. Dose-dependent effects of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials. *Am J Clin Nutr*. 2005;82:806–812.
6. The VITATOPS Trial Study Group. The VITATOPS (VITAmins To Prevent Stroke) trial: Rationale and design of an international, large, simple, randomised trial of homocysteine-lowering multivitamin therapy in patients with recent transient ischemic attack or stroke. *Cerebrovasc Dis*. 2002;13:120–126.
7. The VITATOPS Trial Study Group. The VITAmins TO Prevent Stroke (VITATOPS) trial: Rationale and design of a randomised trial of B-vitamin therapy in patients with recent transient ischemic attack or stroke (NCT00097669) (ISRCTN74743444). *Int J Stroke*. 2007;2:144–150.
8. Fazekas F, Niederkorn K, Schmidt R, Offenbacher H, Horner S, Bertha G, Lechner H. White matter signal abnormalities in normal individuals: correlation with carotid ultrasonography, cerebral blood flow measurements, and cerebrovascular risk factors. *Stroke*. 1988;19:1285–1288.
9. Kapeller P, Barber R, Vermeulen RJ, Adèr H, Scheltens P, Freidl W, Almkvist O, Moretti M, del Ser T, Vaghfeldt P, Enzinger C, Barkhof F, Inzitari D, Erkinjunti T, Schmidt R, Fazekas F; European Task Force of Age Related White Matter Changes. Visual rating of age-related white matter changes on magnetic resonance imaging: scale comparison, interrater agreement, and correlations with quantitative measurements. *Stroke*. 2003;34:441–445.
10. Schmidt R, Enzinger C, Ropele S, Schmidt H, Fazekas F; Austrian Stroke Prevention Study. Progression of cerebral white matter lesions: 6-year results of the Austrian Stroke Prevention Study. *Lancet*. 2003;361:2046–2048.
11. Plummer D. DispImage: a display and analysis tool for medical images. *Revista di Neuroradiologica*. 1992;5:489–495
12. Vermeer SE, van Dijk EJ, Koudstaal PJ, Oudkerk M, Hofman A, Clarke R, Breteler MM. Homocysteine, silent brain infarcts, and white matter lesions: The Rotterdam Scan Study. *Ann Neurol*. 2002;51:285–289.
13. Dufouil C, Alpérovitch A, Ducros V, Tzourio C. Homocysteine, white matter hyperintensities, and cognition in healthy elderly people. *Ann Neurol*. 2003;53:214–221.
14. The VITATOPS Trial Study Group. B vitamins in patients with recent transient ischaemic attack or stroke in the VITAmins TO Prevent Stroke (VITATOPS)trial:arandomised,double-blind,parallel,placebo-controlled trial. *Lancet Neurol*. 2010;9:855–65.
15. Hogervorst E, Ribeiro HM, Molyneux A, Budge M, Smith AD. Plasma homocysteine levels, cerebrovascular risk factors, and cerebral white matter changes (leukoaraiosis) in patients with Alzheimer disease. *Arch Neurol*. 2002;59:787–793.
16. Weir DG, Scott JM. Brain function in the elderly: role of vitamin B12 and folate. *Br Med Bull*. 1999;55:669–682.
17. Lipton SA, Kim WK, Choi YB, Kumar S, D'Emilia DM, Rayudu PV, Arnelle DR, Stamler JS. Neurotoxicity associated with dual actions of homocysteine at the N-methyl-D-aspartate receptor. *Proc Natl Acad Sci USA*. 1997;94:5923–5928.
18. Wolf R, Schabitz FL, Fisher M, Pak HC. The N-methyl-D-aspartate antagonist CNS 1102 protects cerebral gray and white matter from ischemic injury following temporary focal ischemia in rats. *Stroke*. 2003;31:1709–1714
19. Geeganage CM, Diener HC, Algra A, Chen C, Topol EJ, Dengler R, et al. Acute Antiplatelet Stroke Trialists Collaboration Dual or mono antiplatelet therapy for patients with acute ischemic stroke or transient ischemic attack: systematic review and meta-analysis of randomized controlled trials. *Stroke*. 2010:43:1058–1066.
20. Feigin VL, Lawes CM, Bennett DA, Anderson CS. Stroke epidemiology: a review of population-based studies of incidence, prevalence, and case-fatality in the late 20th century. *Lancet Neurol*. 2003;2:43–53.
21. Schmidt R, Petrovic K, Ropele S, Enzinger C, Fazekas F. Progression of leukoaraiosis and cognition. *Stroke*. 2007;38:2619–2625.
22. Vermeulen EG, Stehouwer CD, Valk J, van der Knaap M, van den Berg M, Twisk JW, Prevoo W, Rauwerda JA. Effect of homocysteine-lowering treatment with folic acid plus vitamin B on cerebrovascular atherosclerosis and white matter abnormalities as determined by MRA and MRI: a placebo-controlled, randomized trial. *Eur J Clin Invest*. 2004;34:256–261.
23. Inzitari D, Pracucci G, Poggesi A, Carlucci G, Barkhof F, Chabriat H, Erkinjuntti T, Fazekas F, Ferro JM, Hennerici M, Langhorne P, O'Brien J, Scheltens P, Visser MC, Wahlund LO, Waldemar G, Wallin A, Pantoni L; LADIS Study Group. Changes in white matter as determinant of global functional decline in older independent outpatients: three year follow-up of LADIS (leukoaraiosis and disability) study cohort. *BMJ*. 2009;339:b2477.
24. Debette S, Beiser A, DeCarli C, Au R, Himali JJ, Kelly-Hayes M, Romero JR, Kase CS, Wolf PA, Seshadri S. Association of MRI markers of vascular brain injury with incident stroke, mild cognitive impairment, dementia, and mortality: the Framingham Offspring Study. *Stroke*. 2010;41:600–606.

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

Margherita Cavalieri, Reinhold Schmidt, Christopher Chen, Vincent Mok, Gabriel R. de Freitas, Swithin Song, Qilong Yi, Stefan Ropele, Anja Grazer, Nina Homayoon, Christian Enzinger, Katherine Loh, Ka Sing Lawrence Wong, Adrian Wong, Yunyun Xiong, Hui Meng Chang, Meng Cheong Wong, Franz Fazekas, John W. Eikelboom, Graeme J. Hankey and on behalf of the VITATOPS Trial Study Group
on behalf of the VITATOPS Trial Study Group

Stroke. 2012;43:3266-3270; originally published online October 23, 2012;
doi: 10.1161/STROKEAHA.112.665703
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0039-2499. Online ISSN: 1524-4628

SUPPLEMENTAL MATERIAL

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

	B vitamins (N=174)		Placebo (N=185)		Pval*
	Total†	Value	Total†	Value	
White Matter Hyperintensities					
Deep WMH score					
- 0	165	26 (15.8%)	171	38 (22.2%)	0.444
- 1		59 (35.7%)		54 (31.6%)	
- 2		28 (17.0%)		31 (18.1%)	
- 3		52 (31.5%)		48 (28.1%)	
Periventricular WMH score					
- 0	165	51 (30.9%)	171	58 (33.9%)	0.797
- 1		49 (29.7%)		51 (29.8%)	
- 2		65 (39.4%)		62 (36.3%)	
WMH volume, cm3 (median; range)	165	1.20 (0-65.7)	171	1.67 (0-78.4)	0.485
Lacunes					
Acute lacunes	173	30 (17.3%)	183	31 (16.9%)	0.920
Old lacunes	173	66 (38.1%)	183	64 (34.9%)	0.534
Acute thrombo-embolic infarcts					
Presence of acute thrombo-embolic infarct(s) (N;%)	173	59 (34.1%)	183	67 (36.6%)	0.621
Type of acute infarct (N;%)					
- territorial	59	56 (94.9%)	67	61 (91.0%)	0.400
- watershed	59	3 (5.1%)	67	6 (8.9%)	0.400

Haemorrhages					
Presence of acute haemorrhages (N;%)	174	3 (1.7%)	185	1 (0.5%)	0.286
Old thrombo-embolic infarcts					
Presence of old thrombo-embolic infarct (N;%)	173	40 (23.1%)	183	38 (20.7%)	0.591
Type of old infarct (N;%)					
- territorial	40	39 (97.5%)	38	34 (89.5%)	0.148
- watershed	40	1 (2.5%)	38	4 (10.5%)	0.148
Microbleeds					
Presence of MBs (N;%)	54	36 (66.7%)	52	30 (57.7%)	0.341

*: 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