Antiplatelet therapy and the effects of B vitamins in patients with previous stroke or transient ischaemic attack: a post-hoc subanalysis of VITATOPS, a randomised, placebo-controlled trial

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See Comment page 481

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Summary

Background Previous studies have suggested that any benefits of folic acid-based therapy to lower serum homocysteine in prevention of cardiovascular events might be offset by concomitant use of antiplatelet therapy. We aimed to establish whether there is an interaction between antiplatelet therapy and the effects of folic acid-based homocysteine-lowering therapy on major vascular events in patients with stroke or transient ischaemic attack enrolled in the vitamins to prevent stroke (VITATOPS) trial.

Methods In the VITATOPS trial, 8164 patients with recent stroke or transient ischaemic attack were randomly allocated to double-blind treatment with one tablet daily of placebo or B vitamins (2 mg folic acid, 25 mg vitamin B_6, and 500 μg vitamin B_{12}) and followed up for a median 3·4 years (IQR 2·0–5·5) for the primary composite outcome of stroke, myocardial infarction, or death from vascular causes. In our post-hoc analysis of the interaction between antiplatelet therapy and the effects of treatment with B vitamins on the primary outcome, we used Cox proportional hazards regression before and after adjusting for imbalances in baseline prognostic factors in participants who were and were not taking antiplatelet drugs at baseline and in participants assigned to receive B vitamins or placebo. We also assessed the interaction in different subgroups of patients and different secondary outcomes. The VITATOPS trial is registered with ClinicalTrials.gov, number NCT00097669, and Current Controlled Trials, number ISRCTN74743444.

Findings At baseline, 6609 patients were taking antiplatelet therapy and 1463 were not. Patients not receiving antiplatelet therapy were more likely to be younger, east Asian, and disabled, to have a haemorrhagic stroke or cardioembolic ischaemic stroke, and to have a history of hypertension or atrial fibrillation. They were less likely to be smokers and to have a history of peripheral artery disease, hypercholesterolaemia, diabetes, ischaemic heart disease, and a revascularisation procedure. Of the participants taking antiplatelet drugs at baseline, B vitamins had no significant effect on the primary outcome (488 patients in the B-vitamins group [15%] *vs* 519 in the placebo group [16%]; hazard ratio [HR] 0·94, 95% CI 0·83–1·07). By contrast, of the participants not taking antiplatelet drugs at baseline, B vitamins had a significant effect on the primary outcome (123 in the B-vitamins group [17%] *vs* 153 in the placebo group [21%]; HR 0·76, 0·60–0·96). The interaction between antiplatelet therapy and the effect of B vitamins on the primary outcome was significant after adjusting for imbalance in the baseline variables (adjusted p for interaction=0·0204).

Interpretation Our findings support the hypothesis that antiplatelet therapy modifies the potential benefits of lowering homocysteine with B-vitamin supplementation in the secondary prevention of major vascular events. If validated, B vitamins might have a role in the prevention of ischaemic events in high-risk individuals with an allergy, intolerance, or lack of indication for antiplatelet therapy.

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Introduction

Observational studies show a strong, positive, and dose-related association between serum concentrations of homocysteine and the risk of stroke, which is independent of other vascular risk factors and biologically plausible.[1,2] Homocysteine can be lowered by a mean of 25% (95% CI 23–28) with folic acid supplementation.[3] A meta-analysis of eight randomised, placebo-controlled trials of folic acid supplementation in 37 485 patients[4] showed that, despite yielding an average 25% reduction in homocysteine, folic acid had no significant effect on the rate of first stroke (rate ratio 0·96, 95% CI 0·87–1·06) over a median follow-up of 5 years. However, the role of homocysteine-lowering in stroke prevention might be complex.[5] A meta-analysis of 237 genetic epidemiological studies,[6] in which homocysteine and the presence of the methylene tetrahydrofolate

reductase C677T polymorphism in 60 000 individuals were correlated with 20 885 subsequent stroke events, suggested that established or increasing dietary folate intake in the countries where the trials were undertaken might have modified the effect of lowering homocysteine on risk of stroke.[6]

Antiplatelet therapy might also modify the effect of lowering homocysteine on the risk of stroke and ischaemic heart disease events.[7–9] An exploratory analysis of trials of lowering homocysteine[7] suggested an interaction between antiplatelet therapy and the effect of lowering homocysteine on risk of ischaemic heart disease events: in the five trials with the lowest prevalence of antiplatelet therapy (mean 60%, usually aspirin), the relative risk was 0·93 (95% CI 0·84–1·05) and in the five trials with the highest prevalence (mean 91%) the relative risk was 1·09 (1·00–1·19), p for interaction=0·037. In another analysis of trials of the effects of lowering homocysteine on the risk of stroke events,[8] the effect was greater in the four trials that enrolled patients with renal disease and oesophageal dysplasia (who were not likely to be taking antiplatelet therapy) compared with the trials that enrolled patients with previous vascular disease. The Heart Outcomes Prevention Evaluation 2 (HOPE 2) trial[9] subsequently reported a non-significant trend towards a greater effect of folic acid-based vitamin B supplementation, compared with placebo, in reducing stroke in patients with known cardiovascular disease who were not taking antiplatelet therapy at enrolment compared with patients who were (p for interaction=0·25). The biological plausibility of these findings is supported by the recognised potential for antiplatelet therapy to modify any antithrombotic or other antiatherogenic effects of lowering homocysteine.[10–13]

These analyses prompted us to undertake a post-hoc subanalysis of the vitamins to prevent stroke (VITATOPS) trial. We aimed to explore the hypothesis that there is an interaction between antiplatelet therapy and the effect of folic acid-based vitamin B supplementation on major vascular events in the VITATOPS trial population of patients with previous stroke or transient ischaemic attack.[14]

Methods

Participants

The methods and primary results of the VITATOPS trial have been reported.[14] Briefly, the VITATOPS trial was a randomised, double-blind, parallel, placebo-controlled trial in which 8164 patients were recruited from 123 centres in 20 countries of four continents, and randomly assigned to take one tablet daily of placebo or B vitamins (2 mg folic acid, 25 mg vitamin B_6, 500 µg vitamin B_{12}). Patients were eligible for inclusion if they had a stroke (ischaemic or haemorrhagic) or transient ischaemic attack (eye or brain) within the past 7 months.

Patients were excluded if they were taking folic acid, vitamin B_6, vitamin B_{12}, or a folate antagonist (eg, methotrexate), if they were pregnant or were women of

	Antiplatelet treatment (N=6609)	No antiplatelet treatment (N=1463)	p value
Age (years)	62·9 (12·3)	61·1 (13·2)	<0·0001
Men	4227 (64·0%)	922 (63·0%)	0·4910
Women	2380 (36·0%)	541 (37·0%)	..
Ethnic origin			
White	2755 (43·2%)	511 (36·3%)	<0·0001
East Asian	1455 (22·8%)	445 (31·6%)	..
South Asian	1733 (27·2%)	316 (22·4%)	..
Other	435 (6·8%)	136 (9·7%)	..
Oxfordshire classification of stroke subtype			
Total anterior circulation syndrome	132 (2·0%)	60 (4·1%)	<0·0001
Partial anterior circulation syndrome	3512 (53·7%)	780 (53·9%)	..
Lacunar syndrome	2516 (38·5%)	511 (35·3%)	..
Posterior circulation syndrome	382 (5·8%)	95 (6·6%)	..
Pathological subtype of stroke			
Transient ischaemic attack	1250 (18·9%)	146 (10·0%)	<0·0001
Ischaemic stroke	5117 (77·5%)	574 (39·3%)	..
Intracerebral haemorrhage	82 (1·2%)	654 (44·8%)	..
Subarachnoid haemorrhage	10 (0·2%)	56 (3·8%)	..
Retinal infarction	16 (0·2%)	2 (0·1%)	..
Unknown or uncertain pathology	126 (1·9%)	29 (2·0%)	..
Causal subtype of stroke			
Large artery disease	2788 (42·5%)	227 (15·6%)	<0·0001
Small artery disease	2555 (38·9%)	203 (14·0%)	..
Embolism from the heart	190 (2·9%)	209 (14·4%)	..
Uncertain or unknown	911 (13·9%)	97 (6·7%)	..
Haemorrhagic event	118 (1·8%)	718 (49·4%)	..
Oxford handicap score			
2 or less (independent)	5136 (79·0%)	902 (63·2%)	<0·0001
3 or greater (dependent)	1366 (21·0%)	525 (36·8%)	..
Medical history			
Stroke	1041 (15·8%)	233 (16·1%)	0·7732
Myocardial infarction	501 (7·6%)	95 (6·6%)	0·1797
Peripheral arterial disease	321 (4·9%)	44 (3·0%)	0·0024
Revascularisation procedure of brain, heart, or limbs	482 (7·3%)	82 (5·6%)	0·0219
Hypertension*	4634 (70·3%)	1081 (74·7%)	0·0009
Treated hypertension event	3631 (55·3%)	783 (54·2%)	0·4516
Smoking	3337 (50·7%)	671 (46·4%)	0·0033
Present smoker or at time of event	1615 (24·6%)	288 (19·9%)	0·0001
Hypercholesterolaemia†	2315 (35·2%)	330 (22·9%)	<0·0001
Treated hypercholesterolaemia event	2001 (30·6%)	266 (18·6%)	<0·0001
Diabetes mellitus	1641 (24·9%)	254 (17·5%)	<0·0001
Atrial fibrillation	333 (5·1%)	313 (21·6%)	<0·0001
Ischaemic heart disease	1126 (17·6%)	197 (14·1%)	0·0014
History of depression	451 (7·6%)	92 (7·0%)	0·4947
Alcohol intake (standard drinks [10 g alcohol] per day)	0·8 (2·5)	0·9 (2·5)	0·1888

Data are mean (SD) or n (%). *History of hypertension or treated hypertension at randomisation. †History of hypercholesterolaemia (>6·5 mmol/L) or treated hypercholesterolaemia at randomisation.

Table 1: **Baseline characteristics**

	Antiplatelet treatment (N=6609)		No antiplatelet treatment (N=1463)	
	Placebo group (n=3303)	B-vitamins group (n=3306)	Placebo group (n=729)	B-vitamins group (n=734)
Age (years)	63·0 (12·2)	62·8 (12·4)	61·3 (13·0)	61·0 (13·3)
Men	2097 (63·5%)	2130 (64·4%)	475 (65·2%)	447 (60·9%)
Women	1205 (36·5%)	1175 (35·6%)	254 (34·8%)	287 (39·1%)
Ethnic origin				
White	1378 (43·3%)	1377 (43·1%)	257 (36·5%)	254 (36·1%)
East Asian	732 (23·0%)	723 (22·6%)	217 (30·8%)	228 (32·4%)
South Asian	857 (26·9%)	876 (27·4%)	159 (22·6%)	157 (22·3%)
Other	215 (6·8%)	220 (6·9%)	71 (10·1%)	65 (9·2%)
Oxfordshire classification of stroke subtype				
Total anterior circulation syndrome	71 (2·2%)	61 (1·9%)	31 (4·3%)	29 (4·0%)
Partial anterior circulation syndrome	1758 (53·8%)	1754 (53·6%)	390 (54·1%)	390 (53·8%)
Lacunar syndrome	1256 (38·4%)	1260 (38·5%)	253 (35·1%)	258 (35·6%)
Posterior circulation syndrome	184 (5·6%)	198 (6·0%)	47 (6·5%)	48 (6·6%)
Pathological subtype of stroke				
Transient ischaemic attack	634 (19·2%)	616 (18·7%)	80 (11·0%)	66 (9·0%)
Ischaemic stroke	2560 (77·6%)	2557 (77·4%)	278 (38·2%)	296 (40·4%)
Intracerebral haemorrhage	37 (1·1%)	45 (1·4%)	317 (43·5%)	337 (46·0%)
Subarachnoid haemorrhage	4 (0·1%)	6 (0·2%)	30 (4·1%)	26 (3·5%)
Retinal infarction	9 (0·3%)	7 (0·2%)	2 (0·3%)	0 (0%)
Unknown or uncertain pathology	55 (1·7%)	71 (2·2%)	21 (2·9%)	8 (1·1%)
Causal subtype of stroke				
Large artery disease	1405 (42·9%)	1383 (42·1%)	118 (16·3%)	109 (15·0%)
Small artery disease	1281 (39·1%)	1274 (38·8%)	104 (14·3%)	99 (13·6%)
Embolism from the heart	88 (2·7%)	102 (3·1%)	97 (13·4%)	112 (15·4%)
Uncertain or unknown	453 (13·8%)	458 (13·9%)	54 (7·4%)	43 (5·9%)
Haemorrhagic event	50 (1·5%)	68 (2·1%)	353 (48·6%)	365 (50·1%)
Oxford handicap score				
2 or less (independent)	2556 (78·7%)	2580 (79·2%)	461 (64·8%)	441 (61·6%)
3 or greater (dependent)	690 (21·3%)	676 (20·8%)	250 (35·2%)	275 (38·4%)
Medical history				
Stroke	528 (16·0%)	513 (15·6%)	126 (17·5%)	107 (14·8%)
Myocardial infarction	255 (7·8%)	246 (7·5%)	45 (6·3%)	50 (6·9%)
Peripheral arterial disease	163 (5·0%)	158 (4·8%)	25 (3·5%)	19 (2·6%)
Revascularisation procedure of brain, heart, or limbs	248 (7·5%)	234 (7·1%)	44 (6·0%)	38 (5·2%)
Hypertension*	2330 (70·7%)	2304 (69·9%)	534 (74·0%)	547 (75·4%)
Treated hypertension event	1812 (55·2%)	1819 (55·4%)	390 (54·2%)	393 (54·2%)
Smoking	1669 (50·7%)	1668 (50·6%)	332 (45·9%)	339 (46·9%)
Present smoker or at time of event	806 (24·6%)	809 (24·6%)	138 (19·1%)	150 (20·7%)
Hypercholesterolaemia†	1157 (35·1%)	1158 (35·2%)	161 (22·4%)	169 (23·4%)
Treated hypercholesterolaemia event	987 (30·2%)	1014 (31·0%)	135 (19·0%)	131 (18·2%)
Diabetes mellitus	823 (25·0%)	818 (24·8%)	121 (16·7%)	133 (18·3%)
Atrial fibrillation	165 (5·0%)	168 (5·1%)	152 (21·1%)	161 (22·2%)
Ischaemic heart disease	573 (18·0%)	553 (17·3%)	96 (13·7%)	101 (14·5%)
History of depression	218 (7·3%)	233 (7·8%)	52 (8·0%)	40 (6·1%)
Alcohol intake (standard drinks [10 g alcohol] per day)	0·9 (2·7)	0·8 (2·2)	0·8 (2·2)	1·0 (2·8)

Data are mean (SD) or n (%). *History of hypertension or treated hypertension at randomisation. †History of hypercholesterolaemia (>6·5 mmol/L) or treated hypercholesterolaemia at randomisation.

Table 2: **Baseline characteristics by treatment allocation**

childbearing potential, or if they had a restricted life expectancy (eg, because of ill health).

At enrolment, participants were asked if they were taking antiplatelet drugs (eg, aspirin, clopidogrel, dipyridamole). The trial received ethical approval from national (India, New Zealand, and the UK) and local research ethics committees and all patients provided written informed consent before enrolment.

Procedures

Patients were randomly assigned (1:1) to receive either B vitamins or matching placebo by means of a central 24 h telephone service or an interactive website in which random permuted blocks were stratified by hospital. Treatment groups were masked from patients and investigators. Randomisation was not stratified in accordance with the presence or absence of antiplatelet therapy. The primary outcome was the composite of any stroke, myocardial infarction, or death from vascular causes.

Statistical analysis

We tabulated baseline characteristics and laboratory data in accordance with the presence or absence of antiplatelet therapy at baseline and in accordance with the assigned treatment groups, and expressed them as proportions for categorical variables and means for continuous variables. We compared categorical variables in each group with the χ^2 test, and continuous variables with the t test. We calculated event rates as the number of events during the follow-up period divided by the total number of patients that entered randomisation.

We constructed Kaplan-Meier curves to show the cumulative effects of B vitamins compared with placebo on the primary outcome in participants who were and were not taking antiplatelet therapy at baseline.

We assessed the interaction between antiplatelet therapy and the effects of treatment with B vitamins on the primary outcome by means of Cox proportional hazards regression before and after adjusting for imbalances in important baseline prognostic factors in participants who were and were not taking antiplatelet drugs at baseline, and in participants assigned B vitamins or placebo. We also assessed the consistency of the interaction effect in different subgroups of patients, and in different secondary outcome events including ischaemic stroke, haemorrhagic stroke, myocardial infarction, and death from vascular causes.

We adjusted for certain variables in our models: age, sex, ethnic origin, pathological and causal subtypes of stroke and transient ischaemic attack, stroke severity as measured by the Oxford handicap score, smoking, treated and untreated hypercholesterolaemia, and history of stroke, myocardial infarction, ischaemic heart disease, peripheral arterial disease, atrial fibrillation, and diabetes.

We compared the mean serum concentrations of homocysteine and vitamin B_{12} and mean red-cell

	B-vitamins group		Placebo group		Hazard ratio (95% CI)	p for interaction	Adjusted hazard ratio (95% CI)*	Adjusted p for interaction*
	Total	n (%)	Total	n (%)				
Stroke, myocardial infarction, or vascular death								
Antiplatelet use	3306	488 (14·8%)	3303	519 (15·7%)	0·94 (0·83–1·07)	0·0980	0·98 (0·86–1·11)	0·0204
No antiplatelet use	734	123 (16·8%)	729	153 (21·0%)	0·76 (0·60–0·96)		0·71 (0·55–0·90)	
Stroke								
Antiplatelet use	3306	293 (8·9%)	3303	297 (9·0%)	0·99 (0·84–1·17)	0·0452	1·03 (0·87–1·22)	0·0134
No antiplatelet use	734	65 (8·9%)	729	89 (12·2%)	0·69 (0·50–0·95)		0·65 (0·46–0·91)	
Vascular death								
Antiplatelet use	3306	254 (7·7%)	3303	278 (8·4%)	0·92 (0·78–1·10)	0·0838	0·96 (0·81–1·16)	0·0225
No antiplatelet use	734	70 (9·5%)	729	97 (13·3%)	0·68 (0·50–0·93)		0·63 (0·46–0·88)	
Myocardial infarction								
Antiplatelet use	3306	98 (3·0%)	3303	95 (2·9%)	1·04 (0·78–1·37)	0·6630	0·97 (0·72–1·31)	0·9588
No antiplatelet use	734	18 (2·5%)	729	19 (2·6%)	0·90 (0·47–1·72)		0·89 (0·45–1·79)	
Stroke or vascular death								
Antiplatelet use	3306	453 (13·7%)	3303	476 (14·4%)	0·96 (0·84–1·09)	0·0553	0·99 (0·87–1·14)	0·0072
No antiplatelet use	734	113 (15·4%)	729	145 (19·9%)	0·74 (0·57–0·94)		0·68 (0·52–0·88)	

*Adjusted for age, sex, ethnic origin, history of stroke, myocardial infarction, hypertension, ischaemic heart disease, peripheral arterial disease, diabetes, cholesterol, smoking status, Oxford handicap score, pathology, and cause of stroke and transient ischaemic attack.

Table 3: **Interaction between B-vitamin supplementation and antiplatelet therapy at baseline on each major vascular outcome**

concentration of folate, which were measured at both baseline and follow-up in the same individual, with a paired *t* test. We calculated the difference between baseline and follow-up measures, and tested the interaction effect between antiplatelet use at baseline and treatment allocation with a linear regression model.

We used two-sided significance tests throughout and we deemed a two-sided p value of less than 0·05 to be significant. The VITATOPS trial is registered with ClinicalTrials.gov, number NCT00097669, and Current Controlled Trials, number ISRCTN74743444.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, the writing of the report, or in the decision to submit the paper for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

At baseline, 6609 patients (81%) were in receipt of antiplatelet therapy, 1463 (18%) were not, and in 92 (1%) antiplatelet therapy status was not known. The composite primary outcome of stroke, myocardial infarction, or death from vascular causes was recorded in 616 patients (15%) assigned to receive B vitamins and 678 (17%) assigned to receive placebo (risk ratio 0·91, 95% CI 0·82 to 1·00, p=0·05; absolute risk reduction 1·56%, 95% CI –0·01 to 3·16).[14]

Compared with patients receiving antiplatelet therapy, patients who were not receiving antiplatelet therapy at baseline were more likely to be younger, east Asian, and disabled, to have a haemorrhagic stroke or cardioembolic ischaemic stroke, and to have a history of hypertension or atrial fibrillation (table 1). They were less likely to be smokers and to have a history of peripheral vascular disease, hypercholesterolaemia, diabetes, ischaemic heart disease, and a revascularisation procedure. Of patients who were or were not receiving antiplatelet therapy at baseline, baseline characteristics were evenly distributed between patients assigned to receive either B vitamins or placebo (table 2).

Baseline antiplatelet therapy was an independent significant predictor of a lower rate of subsequent stroke, myocardial infarction, or death from vascular causes in all patients who entered randomisation (hazard ratio [HR] 0·66, 95% CI 0·55–0·81).

Of the 6609 participants in receipt of antiplatelet drugs at baseline, the primary outcome was recorded in roughly 15% of participants assigned to receive B vitamins or placebo (table 3). By contrast, of the 1463 participants who were not in receipt of antiplatelet drugs at baseline, the primary outcome was recorded in slightly more participants in the placebo group (table 3). After adjusting for the effects of imbalance in baseline variables, the HR for the primary outcome for patients assigned B vitamins versus placebo was greater for participants taking antiplatelet therapy than for those who were not (table 3).

The figure shows Kaplan-Meier curves of the cumulative probability of the primary outcome event in patients who were and were not taking antiplatelet at the time of randomisation into the VITATOPS trial. In table 3 we also show the results for the individual components of the primary outcome. The overall results

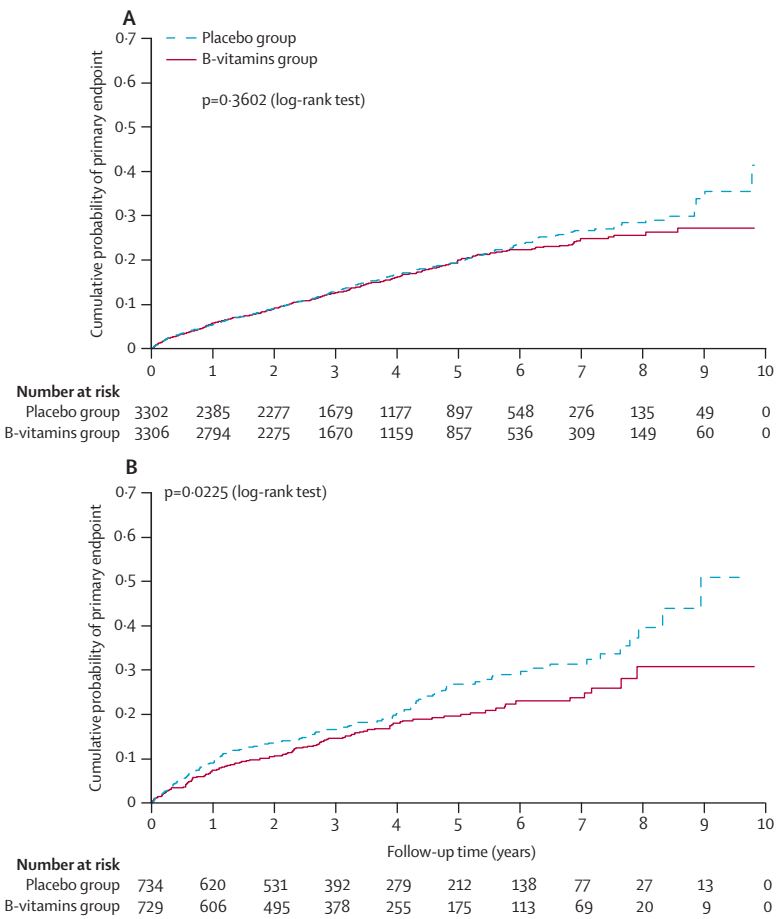

Figure: **Kaplan-Meier curves of the cumulative probability of the primary outcome event**
Cumulative probability of stroke, myocardial infarction, or death from vascular causes in patients with previous stroke or transient ischaemic attack who were (A) or were not (B) in receipt of antiplatelet therapy at the time of randomisation into the VITATOPS trial.

for the primary outcome were consistent for stroke and for vascular death, but not for myocardial infarction.

In table 4 we show a significant interaction between antiplatelet use at baseline and the effect of B vitamins on recurrent ischaemic stroke after adjustment for baseline factors. The trend was similar, but not significant, for recurrent haemorrhagic stroke.

In table 5 we show that of all the listed subgroups, with the exception of participants with cardioembolic ischaemic stroke, the HR for the effect of B vitamins compared with placebo on the primary outcome was lower in patients who were not in receipt of antiplatelet therapy at baseline than in patients who were, but many of the comparisons were not statistically significant.

In table 6 we show that supplementation with B vitamins significantly lowered total homocysteine and increased red cell folate concentration during follow-up in patients who were and were not in receipt of antiplatelet therapy at baseline. Supplementation with B vitamins also significantly increased serum vitamin B_{12} concentration during follow-up in patients in receipt of antiplatelet therapy at baseline, but the effect was not significant for patients not receiving antiplatelet therapy at baseline. The effects of supplementation with B vitamins on lowering total homocysteine and increasing red-cell folate and vitamin B_{12} concentration were not significantly different between patients who were and were not in receipt of antiplatelet therapy at baseline. The p for interaction between antiplatelet therapy at baseline and trial treatment was 0·2501 for total homocysteine, 0·8996 for red cell folate, and 0·6591 for vitamin B_{12}.

After excluding patients with a qualifying diagnosis of haemorrhagic stroke, the interaction between B vitamins and antiplatelet therapy was not significant (adjusted p=0·1159), but the adjusted HR for B vitamins versus placebo on the primary outcome in participants not in receipt of antiplatelet therapy at baseline was still lower (HR 0·75, 95% CI 0·54–1·03) than in participants who were in receipt of therapy (0·98, 0·86–1·12). We also did a matched paired analysis, and a similar pattern was evident.

Discussion

The principal result of the VITATOPS trial was that daily administration of B vitamins to patients with recent stroke or transient ischaemic attack for a median of 3·4 years had no significant effect, compared with placebo, on the overall incidence of major vascular events. However, our post-hoc subanalysis supports hypotheses from previous independent trials of lowering total homocysteine on both ischaemic heart disease and stroke outcome events that antiplatelet therapy, which was taken by most patients, might have modified any favourable effect of folic acid supplementation on major vascular events (panel).[7,9]

The VITATOPS trial had several strengths: systematic bias in treatment allocation was minimised by the randomisation process; observer bias in the assessment of vascular outcomes was minimised by the masking of treatment allocation from assessors, clinicians, and patients; and random error was reduced by the reasonably large number of outcome events. The strengths of our analysis are that it was based on a pre-existing hypothesis (that antiplatelet therapy might interact with the effect of B vitamins on vascular risk), the hypothesis is plausible, the interaction between B-vitamin supplementation and only one subgroup was assessed (antiplatelet use at baseline or not; table 3), the primary trial outcome was the main outcome studied, the distribution of important prognostic factors was reasonably, although not perfectly, balanced between treatment groups within each subgroup (table 2), the analysis was based on appropriate statistical tests of subgroup-treatment effect interaction, all subgroup analyses that were undertaken have been reported, and the results have been interpreted cautiously on the premise that subgroup analyses are intrinsically limited.[15]

	B-vitamins group		Placebo group		Hazard ratio (95% CI)	p for interaction	Adjusted hazard ratio (95% CI)*	Adjusted p for interaction*
	Total	n (%)	Total	n (%)				
All patients†								
Recurrent stroke (ischaemic; first ever or recurrent)								
Antiplatelet use	3306	212 (6·4%)	3303	187 (5·7%)	1·14 (0·94–1·39)	0·1154	1·16 (0·94–1·43)	0·0392
No antiplatelet use	734	36 (4·9%)	729	44 (6·0%)	0·78 (0·50–1·21)		0·69 (0·44–1·11)	
Recurrent stroke (haemorrhagic; first ever or recurrent)								
Antiplatelet use	3306	26 (0·8%)	3303	24 (0·7%)	1·09 (0·63–1·90)	0·0866	1·10 (0·61–1·97)	0·0757
No antiplatelet use	734	15 (2·0%)	729	27 (3·7%)	0·52 (0·28–0·99)		0·48 (0·24–0·94)	
Patients with only non-haemorrhagic stroke or transient ischaemic attack‡								
Recurrent stroke (ischaemic; first ever or recurrent)								
Antiplatelet use	3255	211 (6·5%)	3262	187 (5·7%)	1·14 (0·93–1·38)	0·3208	1·16 (0·94–1·43)	0·1193
No antiplatelet use	371	29 (7·8%)	382	33 (8·6%)	0·88 (0·53–1·44)		0·75 (0·44–1·29)	
Recurrent stroke (haemorrhagic; first ever or recurrent)								
Antiplatelet use	3255	25 (0·7%)	3262	24 (0·7%)	1·05 (0·60–1·84)	0·5815	1·06 (0·59–1·93)	0·6016
No antiplatelet use	371	7 (1·9%)	382	9 (2·4%)	0·76 (0·28–2·05)		0·69 (0·233–2·04)	

*Adjusted for age, sex, ethnic origin, history of stroke, myocardial infarction, hypertension, ischaemic heart disease, peripheral arterial disease, diabetes, cholesterol, smoking status, Oxford handicap score, pathology, and cause of stroke and transient ischaemic attack. †Qualifying event was ischaemic or haemorrhagic stroke or transient ischaemic attack. ‡Qualifying event was only ischaemic stroke or transient ischaemic attack.

Table 4: **Interaction between B-vitamin supplementation and antiplatelet therapy at baseline on recurrent stroke subtypes**

	B-vitamins group		Placebo group		Hazard ratio (95% CI)	p for interaction	Adjusted p for interaction*
	Total	n (%)	Total	n (%)			
Age <60 years							
Antiplatelet use	1237	122 (9·9%)	1208	135 (11·2%)	0·89 (0·70–1·13)	0·4957	0·3521
No antiplatelet use	325	34 (10·5%)	319	43 (13·5%)	0·75 (0·48–1·17)		
Age between 60–69 years							
Antiplatelet use	960	123 (12·8%)	995	139 (14·0%)	0·90 (0·72–1·17)	0·6991	0·4442
No antiplatelet use	206	43 (16·5%)	200	38 (19·0%)	0·83 (0·52–1·32)		
Age >69 years							
Antiplatelet use	1109	243 (21·9%)	1100	245 (22·3%)	1·00 (0·84–1·20)	0·1018	0·0379
No antiplatelet use	203	55 (27·1%)	210	72 (34·3%)	0·73 (0·51–1·03)		
Transient ischaemic attack							
Antiplatelet use	616	63 (10·2%)	634	83 (13·1%)	0·79 (0·57–1·09)	0·2669	0·3513
No antiplatelet use	66	6 (9·1%)	80	17 (21·3%)	0·48 (0·19–1·22)		
Ischaemic stroke							
Antiplatelet use	2557	405 (15·8%)	2560	416 (16·3%)	0·98 (0·85–1·12)	0·5673	0·2114
No antiplatelet use	296	75 (25·3%)	278	73 (26·3%)	0·90 (0·65–1·24)		
Non-haemorrhagic stroke or transient ischaemic attack							
Antiplatelet use	3255	481 (14·8%)	3262	512 (15·7%)	0·94 (0·83–1·07)	0·5907	0·1159
No antiplatelet use	371	82 (22·10%)	382	93 (24·4%)	0·87 (0·65–1·17)		
Intracerebral haemorrhage							
Antiplatelet use	45	6 (13·3%)	37	7 (18·9%)	0·72 (0·24–2·14)	0·5842	0·8060
No antiplatelet use	337	39 (11·6%)	317	57 (18·0%)	0·58 (0·39–0·88)		
Subarachnoid haemorrhage							
Antiplatelet use	6	1 (16·7%)	4	0 (0·0%)	..	..	..
No antiplatelet use	26	2 (7·7%)	30	3 (10·0%)	0·80 (0·13–4·80)		

(Continues on next page)

	B-vitamins group		Placebo group		Hazard ratio (95% CI)	p for interaction	Adjusted p for interaction*
	Total	n (%)	Total	n (%)			
(Continued from previous page)							
Large artery disease							
Antiplatelet use	1383	255 (18·4%)	1405	232 (16·5%)	1·13 (0·95–1·35)	0·2104	0·0438
No antiplatelet use	109	24 (22·0%)	118	31 (26·3%)	0·81 (0·47–1·37)		
Small artery disease							
Antiplatelet use	1274	167 (13·1%)	1281	206 (16·1%)	0·80 (0·65–0·98)	0·5589	0·8683
No antiplatelet use	99	23 (23·3%)	104	33 (31·7%)	0·67 (0·39–1·14)		
Embolism from the heart							
Antiplatelet use	102	22 (21·6%)	88	27 (30·7%)	0·64 (0·37–1·13)	0·1576	0·8186
No antiplatelet use	112	27 (24·1%)	97	21 (21·7%)	1·14 (0·64–2·01)		
Smoking							
Antiplatelet use	1668	279 (16·7%)	1669	275 (16·5%)	1·03 (0·87–1·22)	0·0633	0·0553
No antiplatelet use	339	61 (18·0%)	332	81 (24·4%)	0·73 (0·52–1·02)		
No smoking							
Antiplatelet use	1626	206 (12·7%)	1623	242 (14·9%)	0·84 (0·70–1·01)	0·7663	0·1333
No antiplatelet use	384	61 (15·9%)	391	71 (18·2%)	0·80 (0·57–1·13)		
Diabetes							
Antiplatelet use	818	147 (18·0%)	823	153 (18·6%)	1·00 (0·79–1·25)	0·0555	0·0205
No antiplatelet use	133	32 (24·1%)	121	43 (35·5%)	0·61 (0·39–0·97)		
No diabetes							
Antiplatelet use	2480	339 (13·7%)	2471	364 (14·7%)	0·93 (0·80–1·08)	0·3179	0·1520
No antiplatelet use	593	89 (15·0%)	602	109 (18·1%)	0·80 (0·60–1·06)		
High cholesterol (≥6·5 mmol/L)							
Antiplatelet use	1158	170 (14·7%)	1157	184 (15·9%)	0·92 (0·75–1·14)	0·3346	0·2473
No antiplatelet use	169	29 (17·2%)	161	36 (22·4%)	0·72 (0·44–1·18)		
Normal cholesterol (<6·5 mmol/L)							
Antiplatelet use	1496	214 (14·3%)	1463	209 (14·3%)	1·03 (0·85–1·25)	0·0265	0·0069
No antiplatelet use	354	51 (14·4%)	377	75 (19·9%)	0·66 (0·46–0·94)		
Treated high cholesterol							
Antiplatelet use	1014	144 (14·2%)	987	160 (16·2%)	0·88 (0·70–1·10)	0·1825	0·2762
No antiplatelet use	131	22 (16·8%)	135	25 (25·2%)	0·60 (0·35–1·03)		
Untreated high cholesterol							
Antiplatelet use	2261	339 (15·0%)	2283	356 (15·6%)	0·97 (0·84–1·13)	0·2154	0·0234
No antiplatelet use	589	97 (16·5%)	577	114 (19·8%)	0·81 (0·62–1·07)		

*Adjusted for age, sex, ethnic origin, history of stroke, myocardial infarction, hypertension, ischaemic heart disease, peripheral arterial disease, diabetes, cholesterol, smoking status, Oxford handicap score, pathology, and cause of stroke and transient ischaemic attack.

Table 5: **Interaction between B-vitamin supplementation and antiplatelet therapy at baseline on the primary outcome stratified by baseline characteristics**

Potential limitations are that, because this substudy was not a primary aim or prespecified analysis of the VITATOPS trial, the type of antiplatelet therapy taken (eg, aspirin, clopidogrel, aspirin combined with dipyridamole) was not recorded, and there was a significant imbalance in baseline characteristics of participants in receipt of antiplatelet therapy compared with participants who were not (table 1), and a mild imbalance in baseline characteristics in participants assigned to receive B vitamins versus placebo (table 2). The more favourable recorded effect of B vitamins in participants not in receipt of antiplatelet therapy might have been confounded by the reason they were not in receipt of the therapy—ie, B vitamins might have been more effective in patients of east Asian origin or patients with cardioembolic ischaemic stroke or intracerebral haemorrhage (who tend not be given antiplatelet drugs). However, we adjusted for the effects of this imbalance on the rates of each vascular outcome in our Cox multiple regression analysis. Through our Cox analysis we identified that, after adjusting for these effects, the use of antiplatelet therapy at baseline was a significant, independent predictor of the incidence of major vascular events ($p<0·0001$) and that there was a significant interaction between antiplatelet therapy and treatment with B vitamins on the primary outcome (adjusted p for

	Antiplatelet treatment			No antiplatelet treatment		
	Baseline	Follow-up	Difference (95% CI); p value*	Baseline	Follow-up	Difference (95% CI); p value*
Homocysteine (μmol/L)						
B-vitamins group	13·7 (6·6)	10·5 (4·4)	−3·18 (−2·66 to −3·70); p<0·0001	12·4 (4·3)	9·9 (2·6)	−2·46 (−1·46 to −3·46); p<0·0001
Placebo group	13·4 (4·9)	14·4 (5·8)	0·94 (0·40 to 1·47); p=0·0006	13·3 (5·8)	13·8 (5·1)	0·56 (−0·50 to 1·63); p=0·2937
Red cell folate (nmol/L)						
B-vitamins group	971·6 (464·6)	2297·9 (789·4)	1326·2 (1195·8 to 1456·6); p<0·0001	906·8 (432·7)	2090·9 (752·3)	1184·1 (951·1 to 1417·2); p<0·0001
Placebo group	867·0 (445·3)	1156·5 (686·0)	289·5 (186·9 to 392·1); p<0·0001	990·7 (515·9)	1112·9 (628·7)	122·2 (−109·2 to 353·5); p=0·2901
Vitamin B_{12} (pmol/L)						
B-vitamins group	312·3 (139·3)	367·5 (195·6)	55·1 (21·3 to 89·0); p=0·0016	368·6 (192·4)	396·9 (239·4)	28·4 (−67·0 to 123·7); p=0·5494
Placebo group	311·9 (127·5)	205·7 (132·2)	−106·2 (−79·7 to −132·8); p<0·0001	342·7 (127·5)	186·4 (90·7)	−156·3 (−109·8 to −202·8); p<0·0001

Data are mean (SD) unless otherwise stated. *Comparison between baseline and during the follow-up was undertaken with a paired *t* test. Some of the follow-up measures were taken during follow-up (eg, at the regular follow-up assessments every 6 months) and some at the end of follow-up.

Table 6: **Homocysteine, red cell folate, and vitamin B_{12} concentrations at baseline and during follow-up**

interaction=0·0204), stroke (adjusted p for interaction=0·0134), and death from vascular causes (adjusted p for interaction=0·0225). We acknowledge the possibility of residual imbalance in other, unmeasured, prognostic factors at baseline, for which we could not adjust our analysis, and that such residual confounding after adjusting for imbalances in measured prognostic factors (eg, haemorrhagic stroke, cardioembolic ischaemic stroke) could affect our results. We also acknowledge that our findings might represent not an interaction of B-vitamin supplementation with antiplatelet therapy but a significant effect of lowering homocysteine by B-vitamin supplementation in patients with haemorrhagic stroke or cardioembolic ischaemic stroke.

If our findings are valid, the mechanisms by which raised homocysteine might impair vascular function in the absence of antiplatelet therapy remain to be ascertained. Laboratory investigations suggest several potential mechanisms, including impairment of endothelial function, oxidation of low-density lipids, increased monocyte adhesion to the blood vessel wall, increased lipid uptake and retention, activation of inflammatory pathways, stimulatory effects on smooth-muscle-cell proliferation, and prothrombotic tendency mediated by activation of coagulation factors and platelet dysfunction.[11–13] If antiplatelet therapy really does modify the effects of lowering homocysteine on vascular outcomes, this might be mediated by direct effects of antiplatelet drugs on platelet activation and thrombus formation, or indirect effects of antiplatelet drugs, such as aspirin, in reducing vasoconstrictor tone, vascular smooth-muscle-cell proliferation, and release of inflammatory cytokines, oxygen radicals, and growth factors.[10]

In conclusion, our findings of a significant interaction between antiplatelet therapy and the effect of B vitamins on the primary outcome, in our exploratory analysis of an independent group of patients with previous stroke or transient ischaemic attack, support the hypothesis generated from other studies that antiplatelet therapy might modify any potential benefits of lowering homocysteine with folic-acid supplementation in the secondary prevention of major vascular events. Rather than antiplatelet therapy negating all of the effects of lowering homocysteine, it is also possible that lowering homocysteine might have a small benefit independent of antiplatelet therapy and a larger benefit in the absence of additional prophylactic antiplatelet therapy.

The external validity of our findings can be assessed more reliably by means of a meta-analysis of the relevant data from all individual patients enrolled in

Panel: Research in context

Systematic review

We searched PubMed with the terms "homocysteine", "folic acid", "vitamins", "antiplatelet", "aspirin", "clopidogrel", "dipyridamole", "cilostazol", "stroke", "ischaemic heart disease", "major vascular events", "interaction", "randomised trial", and "clinical trial" for reports of an interaction between antiplatelet therapy and treatments that lower homocysteine in the prevention of stroke and other major vascular events. We searched for work published before March, 2012. The quality of evidence we required was a randomised, controlled trial or meta-analysis of such trials. We identified the Heart Outcomes Prevention Evaluation 2 trial[9] and the meta-analysis of randomised trials of lowering total homocysteine on risk of ischaemic heart disease events[7] as directly relevant, and a further meta-analysis[8] as indirectly relevant.

Interpretation

The results of our exploratory analyses of the VITATOPS trial support previous hypotheses that antiplatelet therapy, which was taken by most patients, might modify any favourable effect of folic acid supplementation on major vascular events.[7,9] If our finding are validated in independent studies, B vitamins might have a role in the prevention of vascular events in high-risk individuals with an allergy, intolerance, or lack of indication for antiplatelet therapy, such as those with haemorrhagic stroke.

trials of B vitamins to prevent both stroke and ischaemic heart disease events. If validated, the implications of the findings for clinicians are that B vitamins might have a role in the prevention of vascular events in individuals at high risk but who have an allergy to, intolerance of, or lack of indication for antiplatelet therapy, such as those who are also at risk of bleeding events (eg, haemorrhagic stroke).

Contributors

GJH initiated the analysis for this substudy and wrote the first and final drafts of the report. QY did all the analyses. JWE, KRL, CC, DX, JCN, UKR, WU, SR, JG, and RS contributed to each draft of the report. All authors were members of the International Steering Committee of the VITATOPS trial.

Conflicts of interest

We declare that we have no conflicts of interest.

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