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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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 B₆, and 500 μg vitamin B₁₂) 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] 0·96, 95% CI 0·87–1·06) over a median follow-up of 5 years. However, the role of homocysteine-lowering therapy with previous stroke or transient ischaemic attack: a post-hoc subanalysis of VITATOPS, a randomised, placebo-controlled trial

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reductase C677T polymorphism in 60000 individuals were correlated with 20885 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.4

Antiplatelet therapy might also modify the effect of lowering homocysteine on the risk of stroke and ischaemic heart disease events.5,6 An exploratory analysis of trials of lowering homocysteine7 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) trial9 subsequently reported a non-significant trend towards a greater effect of lowering homocysteine on risk of stroke (mean 60%, usually aspirin), the relative risk was 0.94 (95% CI 0.79–1.13), p for interaction=0.125. These findings are supported by the recognised potential for antiplatelet therapy to modify any antithrombotic or other antithromogenic 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.15 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 B6, 500 μg vitamin B12). 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 B12, vitamin B6, or a folate antagonist (eg, methotrexate), if they were pregnant or were women of Childbearing age. 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.

Table 1: Baseline characteristics

| Baseline characteristic | 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 (42.3%)                   | 511 (35.3%)                     | <0.0001 |
| East Asian              | 1455 (22.8%)                   | 445 (31.6%)                     |         |
| South Asian             | 1733 (27.1%)                   | 316 (22.4%)                     |         |
| Other                   | 435 (6.8%)                     | 135 (9.7%)                      |         |
| Oxford 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         | 5172 (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†   | 2335 (35.2%)                   | 330 (22.9%)                     | <0.0001 |
| Treated hypercholesterolaemia event | 2001 (30.6%)            | 256 (18.6%)                     | <0.0001 |
| Diabetes mellitus        | 1641 (24.4%)                   | 254 (17.5%)                     | <0.0001 |
| Atrial fibrillation      | 332 (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           | 0.8 (0.5%)                     | 0.9 (0.5%)                      | 1.0888  |

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

| Age (years) | Placebo group (n=3303) | B-vitamins group (n=3306) | Placebo group (n=729) | B-vitamins group (n=734) |
|-------------|------------------------|---------------------------|-----------------------|--------------------------|
| Men         | 63.0 (12.2)            | 62.8 (12.4)               | 61.3 (13.0)           | 61.0 (13.3)              |
| 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%)                 | 37 (4.5%)             | 33 (4.6%)                |
| 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%)              | 97 (13.4%)            | 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%)           | 360 (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%)              | 323 (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 (35.3%)      | 1158 (35.2%)              | 161 (22.4%)           | 169 (23.4%)              |
| Treated hypercholesterolaemia event | 987 (29.2%)       | 1014 (31.0%)              | 125 (17.5%)           | 131 (18.2%)              |
| Diabetes mellitus | 823 (25.0%)        | 818 (24.8%)               | 121 (16.7%)           | 123 (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 (7.8%)             | 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.
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 0·83–1·00, p=0·0980; absolute risk reduction 1·56%, 95% CI –0·01 to 3·16%).

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 therapy at baseline, baseline characteristics were evenly distributed between patients assigned to receive either B vitamins or placebo (table 2).

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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 therapy 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

| B-vitamins group | Placebo group | Hazard ratio (95% CI) | p for interaction | Adjusted hazard ratio (95% CI)* | Adjusted p for interaction* |
|------------------|---------------|----------------------|------------------|-------------------------------|-----------------------------|
| 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) | 0·0392 | 0·71 (0·55–0·90) | 0·0254 |
| 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) | 0·1439 | 0·65 (0·46–0·91) | 0·0254 |
| 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) | 0·0160 | 0·61 (0·46–0·88) | 0·0001 |
| 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 (0·67–1·22) | 0·6939 | 0·89 (0·54–1·47) | 0·6939 |
| 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) | 0·0019 | 0·68 (0·52–0·88) | 0·0001 |

*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.**
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 B₁₂ 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 B₁₂ 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 B₁₂.

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).

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 trial treatment; 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.¹⁷
### 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 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                     | 0·16 (0·94–1·43)            | 0·0392                      |
| No antiplatelet use | 734           | 36 (4·9%)             | 729              | 44 (6·0%)                       | 0·78 (0·59–1·21)            | 0·96 (0·69–1·11)            |
| 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)            |
| **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.** | |                       |                  |                                 |                            |
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 to 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 interaction).
Inflammatory pathways, stimulatory effects on smooth-muscle-cell proliferation, and prothrombotic tendency mediated by activation of coagulation factors and platelet dysfunction. Aspirin, in reducing vasocconstrictor tone, vascular smooth-muscle-cell proliferation, and release of inflammatory cytokines, oxygen radicals, and growth factors.

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

| Homocysteine (μmol/L) | Baseline | Follow-up | Difference (95% CI); p value* |
|-----------------------|----------|-----------|-----------------------------|
| B-vitamins group      | 311·9 (127·5) | 367·5 (195·6) | –55·6 (–79·7 to –31·5); p<0·0001 |
| Placebo group         | 311·9 (127·5) | 367·5 (195·6) | –55·6 (–79·7 to –31·5); 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 B12 concentrations at baseline and during follow-up.
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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