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 Ischaemic Attack or Stroke

The VITATOPS Trial Study Group

Key Words
Homocysteine · Multivitamins · Stroke · Transient ischaemic attack · VITATOPS Trial

Abstract
Background: Epidemiological studies suggest that raised plasma concentrations of total homocysteine (tHcy) may be a common, causal and treatable risk factor for atherothromboembolic ischaemic stroke. Although tHcy can be lowered effectively with small doses of folic acid, vitamin B12 and vitamin B6, it is not known whether lowering tHcy, by means of multivitamin therapy, can prevent stroke and other major atherothromboembolic vascular events. Purpose: To determine whether vitamin supplements (folic acid 2 mg, B6 25 mg, B12 500 µg) reduce the risk of stroke, and other serious vascular events, in patients with recent stroke or transient ischaemic attacks of the brain or eye (TIA). Methods: An international, multi-centre, randomised, double-blind, placebo-controlled clinical trial. Results: As of November 2001, more than 1,400 patients have been randomised from 10 countries in four continents. Conclusion: VITATOPS aims to recruit and follow up 8,000 patients between 2000 and 2004, and provide a reliable estimate of the safety and effectiveness of dietary supplementation with folic acid, vitamin B12, and vitamin B6 in reducing recurrent serious vascular events among a wide range of patients with TIA and stroke.

Introduction
Stroke is looming as an increasing public health problem [1–3]. Potential strategies for reducing the burden of stroke include primary prevention, treatment of acute stroke, and continuing care for survivors of previous stroke or transient ischaemic attacks of the brain or eye (TIA). Despite the availability of proven strategies for prevention of recurrent stroke [4], its incidence remains high – 9% (95% confidence interval, CI: 5.4–12.1%) in the first 6 months after stroke, and 23% (95% CI: 16.8–28.1%) over 5 years [5]. Reasons include inadequate application of effective strategies of stroke prevention, and failure to recognise and treat other, as yet unknown, causal risk factors for stroke. At present, only two thirds of all episodes of ischaemic stroke can be attributed to known genetic and environmental factors [6]. There is now a large body of evidence suggesting that an elevated plasma total homocysteine concentration (tHcy) is a common and causal risk factor for atherosclerotic ischaemic stroke [7–10].

Three recent systematic reviews of observational studies [7, 9, 10] reveal an independent relationship between higher concentrations of tHcy in individuals with cerebral, coronary, and peripheral arterial disease compared...
with individuals without vascular disease. Furthermore, high tHcy is associated specifically with ischaemic stroke caused by large-artery disease, and less so small-artery disease, but not with cardio-embolic or other non-atherosclerotic causes of stroke [11]. The association between elevated tHcy and atherosclerotic disease is also dose-related, strong, biologically plausible and supported by experimental studies [12–16]. However, the results obtained by different epidemiological methods are inconsistent. In addition, the temporal relationship between the onset of elevated tHcy and the onset of stroke is unclear; the finding of a stronger association in case-control studies than cohort studies suggests that elevated tHcy may be an acute-phase reactant that rises or falls after the stroke or other vascular events in response to tissue damage or tissue repair [10, 17–20]. Furthermore, the relationship between a polymorphism in methylenetetrahydrofolate reductase (MTHFR; C677T), which is associated with high tHcy in the plasma, and increased cardiovascular risk is not clear [21, 22]. The best evidence that the epidemiological and statistical association between plasma tHcy and arterial disease is causal would come from large randomised controlled trials showing unambiguously that lowering the level of plasma tHcy leads to a reduction in the incidence of major vascular events [23].

It is possible to lower plasma tHcy by 25% (95% CI: 23–28%) with folic acid 0.5–5 mg daily, and by a further 7% (95% CI: 3–10%) with vitamin B\textsubscript{12} 0.02–1 mg daily.

### Table 1. Summary of the randomised trials of homocysteine-lowering therapy with vitamins on surrogate outcome measures of vascular disease\textsuperscript{a}

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Population</th>
<th>n</th>
<th>Intervention</th>
<th>Design</th>
<th>Surrogate outcome</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peterson et al. [29] (1998)</td>
<td>carotid atherosclerosis; tHcy &gt; 14 μmol/l</td>
<td>38</td>
<td>folic acid + vitamin B\textsubscript{6} + vitamin B\textsubscript{12}\textsuperscript{b}</td>
<td>uncontrolled ‘before-after’ study</td>
<td>rate of progression of carotid plaque area</td>
<td>vitamin therapy associated with reduction in rate of progression of plaque area</td>
</tr>
<tr>
<td>Woo et al. [30] (1999)</td>
<td>healthy volunteers; tHcy &gt; 75th percentile</td>
<td>17</td>
<td>folic acid vs. placebo</td>
<td>randomized double-blind crossover trial</td>
<td>flow-mediated EDV of the brachial artery</td>
<td>folic acid significantly increased endothelium-dependent flow</td>
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<tr>
<td>Verhaar et al. [31] (1998)</td>
<td>FH vs. healthy matched controls</td>
<td>20</td>
<td>5-MTHF</td>
<td>controlled observational study</td>
<td>EDV of forearm vessels</td>
<td>5-MTHF: restored impaired forearm flow in FH, no effect in controls</td>
</tr>
<tr>
<td>Bellamy et al. [32] (1999)</td>
<td>healthy volunteers, tHcy &gt; 13 μmol/l</td>
<td>18</td>
<td>folic acid vs. placebo</td>
<td>randomized double-blind crossover trial</td>
<td>EDV of forearm vessels</td>
<td>folic acid significantly enhances endothelium-dependent vascular function</td>
</tr>
<tr>
<td>Verhaar et al. [33] (1999)</td>
<td>FH</td>
<td>20</td>
<td>folic acid vs. placebo</td>
<td>randomized double-blind crossover trial</td>
<td>EDV of forearm vessels</td>
<td>folic acid: restored endothelium-dependent flow; placebo: no effect</td>
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<tr>
<td>Wilmink et al. [34] (2000)</td>
<td>healthy volunteers</td>
<td>20</td>
<td>folic acid vs. placebo</td>
<td>randomized double-blind trial</td>
<td>EDV of forearm vessels following an acute fat load</td>
<td>folic acid pre-treatment prevented lipid-induced reduction in EDV</td>
</tr>
<tr>
<td>Title et al. [35] (2000)</td>
<td>angiographic coronary artery disease; tHcy &gt; 9 μmol/l</td>
<td>75</td>
<td>placebo vs. folic acid vs. folic acid + vitamin C/E</td>
<td>randomized double-blind trial</td>
<td>EDV of forearm vessels</td>
<td>folic acid alone but not folic acid plus vitamin C/E significantly improved endothelium-dependent flow</td>
</tr>
<tr>
<td>Hackam et al. [36] (2000)</td>
<td>carotid atherosclerosis</td>
<td>101</td>
<td>folic acid + vitamin B\textsubscript{6} + vitamin B\textsubscript{12}\textsuperscript{b}</td>
<td>uncontrolled ‘before-after’ study</td>
<td>rate of progression of carotid plaque area</td>
<td>vitamin therapy associated with reduction in rate of progression of plaque area</td>
</tr>
<tr>
<td>Vermeulen et al. [37] (2000)</td>
<td>healthy siblings of patients with premature atherothrombotic disease</td>
<td>167</td>
<td>folic acid + B\textsubscript{6} vs. placebo</td>
<td>randomized double-blind trial</td>
<td>subclinical atherosclerosis measured by exercise ECG, ABI, carotid and femoral U/S</td>
<td>folic acid + B\textsubscript{6} reduced rate of abnormal exercise ECG</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Does not include studies in patients with renal failure.

\textsuperscript{b} Folic acid alone prior to 1996.
The addition of vitamin B₆ to folic acid may also lower plasma tHcy [24–28].

Randomised controlled trials have shown that lowering tHcy by these means improves surrogate markers of cardiovascular disease (table 1) [29–37], but it remains unknown if lowering homocysteine prevents ‘hard’ clinical vascular events. A number of trials investigating the effect of lowering tHcy on stroke, myocardial infarction and vascular death are now in progress [8]. Two trials are studying patients with TIA and stroke: the Vitamins in Stroke Prevention Trial (Bowman Gray School of Medicine, USA) [38] and the VITATOPS Study (Royal Perth Hospital, University of Western Australia) [39]. This paper describes the design of the VITATOPS Trial.

Objectives of the VITATOPS Trial

The primary objective of the VITATOPS trial is to determine, in a randomised, double-blind, placebo-controlled trial, whether the addition of vitamin supplements (folic acid 2 mg, B₆ 25 mg, B₁₂ 500 µg) to best medical and surgical management (including modification of risk factors) reduces the combined incidence of recurrent non-fatal serious vascular events (stroke and MI) and death due to vascular causes in a wide variety of patients with recent stroke or TIA.

Secondary objectives concern the impact of these vitamin supplements on the incidence of: TIA, peripheral arterial disease as measured by leg amputation, unstable angina, revascularization procedures, depression, and dementia; and the consistency of the primary effect of treatment in subgroups of patients defined by race, genotype, and pathological and aetiological subtype of stroke.

The administrative structure of the trial is shown in the Appendix.

Patients

Inclusion Criteria

All patients presenting within 7 months of stroke (ischaemic or haemorrhagic) or TIA (eye or brain), as defined by standard criteria [40], are eligible. The ‘cut-off’ of 7 months was chosen in order to maximise the rate of vascular events, and also patient recruitment; the risk of recurrent vascular events of the brain is highest within the first few months after TIA or stroke, and patients who have been entered into randomised trials of acute treatments for stroke generally require a 6-month period of follow-up that also demands exclusion from participation in other trials during that time. After the 6-month follow-up, such patients can then be recruited into VITATOPS. In addition, the patient must: agree to take study medications, be geographically accessible for follow-up and provide written informed consent.

Patients with haemorrhagic stroke have been included because they are more commonly hypertensive and smokers (than controls), and therefore at increased risk of ischaemic vascular events which may be reduced by lowering tHcy. It is also important to determine whether any effect of lowering tHcy is consistent among pathological (as well as aetiological) subtypes of stroke. Furthermore, their inclusion maximises simplicity of recruitment. However, because the case fatality of haemorrhagic stroke is high, the proportion of these patients in VITATOPS will be low and any effect on statistical power limited.

Exclusion Criteria

Patients taking folic acid or vitamin B₆ on medical advice; taking methotrexate for any reason; pregnant patients or women of child-bearing potential who are at risk of pregnancy, and patients with a limited life expectancy were excluded from the study.

Baseline Data

Table 2 lists the data that are to be collected at baseline.

Randomisation

After baseline data have been provided by the investigator and it is confirmed that the patient meets the study eligibility criteria, a central 24-hour telephone service or an interactive website (www.health.wa.gov.au/VITATOPS/) uses random permuted blocks stratified by hospital to allocate a treatment pack number, the tablets being either vitamin supplements (folic acid 2 mg, B₆ 25 mg, B₁₂ 500 µg) or matching placebo.

Treatment begins as soon as possible after randomisation. The tablets may be taken orally or in crushed form via a nasogastric feeding tube.

Follow-Up

Follow-up of all patients is scheduled for every 6 months after randomisation until completion of the trial. Each follow-up visit or phone call records major outcome
<table>
<thead>
<tr>
<th>Table 2. Data collection at baseline¹ and follow-up</th>
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<tr>
<td>Consent</td>
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<tr>
<td>Clinical history &amp; examination</td>
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<tr>
<td>Enrolment form &amp; baseline data</td>
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<td>CT scan</td>
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<td>Carotid Doppler studies</td>
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<td>Creatinine</td>
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<td>Fasting glucose &amp; lipids</td>
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<td>Follow-up for outcome events</td>
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<td>Adverse events</td>
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<td>HAD Scale</td>
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<td>MMSE</td>
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¹ In the interests of making the trial as simple as possible, and because the relationship between vascular disease and tHcy is monotonically continuous, neither measurement of tHcy or a particular level of tHcy is a criterion for enrolment.

Consent
The VITATOPS Trial

Clinical history & examination

Enrolment and randomisation

Follow-up for outcome events

Adverse events

HAD Scale

MMSE

CT scan

Carotid Doppler studies

Creatinine

Fasting glucose & lipids

Events, adverse events, use of vitamins and compliance with trial medication (questioning, tablet count, return of tablet container), measures to prevent recurrent stroke (advice on smoking and blood pressure, anti-hypertensive therapy, aspirin therapy, lipid-lowering therapy, anti-diabetic medications, anticoagulation), cardiovascular surgery (carotid, coronary, aortic, peripheral arterial) or angioplasty, treatment for depression, and diagnosis of dementia since the last appointment.

Trial medication may be discontinued at the patient’s request or in the setting of a serious adverse event. However, all patients continue under follow-up for outcome events or death from any cause.

Temporary interruption of the trial medication, for example because of surgery, is reported at the next follow-up visit.

Outcomes

The primary outcome is the composite event ‘stroke, MI, or death from any vascular cause’, whichever occurs first. Each is defined in the study protocol, as are adverse events that are to be recorded.

Secondary outcomes include TIA, depression [41], dementia [42], unstable angina, revascularization procedures, and amputation of a leg for peripheral arterial disease.

Every outcome and serious adverse event is audited by a blinded Events Adjudication Committee (see Appendix). The blinded Data Monitoring and Safety Committee will review all validated serious adverse events and report to the Steering Committee.

Statistical Analysis

We plan for intervention and placebo groups of equal size, a minimum follow-up of 1 year for the last patient randomised, an annual primary outcome event rate of 8% in the placebo group [43], and a 15% relative reduction in risk of the primary outcome event among patients allocated the multivitamin intervention (i.e. 6.8%/year). Using a type I error of 5% and type II error of 20%, and assuming an average follow-up of 2 years, a sample size of 3,982 patients would be required in each group (table 3). We aim to randomise 8,000 patients by the end of 2003, and complete final follow-up at the end of 2004. However,
the Steering Committee will be flexible in dictating the need for ongoing recruitment and continuing follow-up, depending on the overall rate of the primary outcome event in the entire cohort at each interim analysis.

**Interim Analyses**

The Data Monitoring and Safety Committee will conduct interim analyses after follow-up of 5,000 and 10,000 person years. The analysis at each of these intervals will focus on the primary composite outcome using an intention-to-treat approach unadjusted for covariate imbalances and applied to the active and control treatment groups as a whole. The Data Monitoring and Safety Committee will be blind to the treatment allocation.

**Final Analysis**

The final analysis will compare the incidence of the primary outcome event between the vitamin and placebo treatment groups, using the intention-to-treat principle. Events will only be included in a supplementary on-treatment analysis if they occur during or within 28 days of discontinuation of the medication. Kaplan-Meier curves will be used for graphic comparison of survival free of a primary endpoint and will be compared using a two-sided Mantel-Haenszel test. Cox’s proportional hazards models will be used to adjust for differences in baseline prognostic variables.

In order to examine the consistency of treatment effect, the following subgroup analyses are planned (with the primary study outcome as the dependent variable): age, sex, race, clinical syndrome pathology (TIA, ischaemic stroke, haemorrhagic stroke), pathogenesis (ischaemic stroke due to large-artery disease, small-artery disease, embolism from the heart, and other causes), stroke severity (Oxford Handicap Score ≤ 2, ≥ 3), and MTHFR genotype.

### Substudies

**Homocysteine in Stroke Study (Principal Investigator: Dr. Ross Baker, Australia)**

The Homocysteine in Stroke Study is a 400-patient blood substudy designed to evaluate the impact of homocysteine-lowering therapy on plasma tHcy and circulating blood markers of endothelial and haemostatic function in order to elucidate further the mechanisms by which homocysteine-lowering therapy may have a beneficial effect (if proven to do so). Fasting blood samples will be collected at baseline and 6 months after randomisation for measurement of blood concentrations of E-selectin (a specific marker of endothelial dysfunction), von Willebrand factor (a marker of both endothelial dysfunction and platelet activation), fibrinogen, P-selectin (a marker of platelet activation) and plasma tHcy.

**MRI Substudy (Principal Investigator: Dr. Reinhold Schmidt, Austria)**

The MRI substudy aims to determine whether vitamin supplementation with subsequent reduction of homocysteine concentrations slows the progression of small-vessel disease in the brain and delays or prevents the occurrence of clinical and MRI brain scan evidence of vascular dementia in more than 600 patients with TIA or stroke who do not have dementia at the time of entry into the study [44]. Analysis of the baseline and follow-up MRI scans will be performed centrally, in Graz, Austria, by a single experienced reader using a standardized assessment protocol for presence of white matter hyperintensities [45]. The scans will be read blinded to the clinical data.
**Ethical Aspects**

This trial is being performed in agreement with the Declaration of Helsinki. After verification of the inclusion and exclusion criteria, patients are asked to provide written informed consent according to the requirements of the institutional ethics committee at each participating centre.

**Discussion**

Elevated plasma concentrations of tHcy above 15 μmol/l are present in less than 5% of the general population but as many as 50% of patients with stroke (and other atherothrombotic vascular diseases) [11]. However, it remains uncertain whether high tHcy is a causal risk factor for stroke, and should be lowered.

Plasma concentrations of tHcy can be reduced effectively by folic acid, vitamin B6 and vitamin B12 supplementation, and controlled trials have shown some beneficial effects on markers of vascular function. However, these markers are not established vascular risk factors or valid predictors of ‘hard’ vascular events.

The VITATOPS study is an international multi-centre study, involving patients of many different backgrounds who, with the exception of residents in the USA, live in countries where folate fortification is not mandatory. It is therefore a unique project because of the diversity of its study population, simplicity, and proposed size, and the results should have world-wide applicability. Furthermore, if the study does show that multivitamin therapy, by means of folic acid, vitamin B6, and vitamin B12, is safe and effective in preventing major serious vascular events among patients with TIA and stroke, then it could have a major impact in reducing the burden of stroke, the second most common single cause of death in the world, by a simple, affordable, and practical measure.

However, until it has been shown that multivitamin therapy reduces the rate of recurrent stroke and other serious vascular events in patients with prior stroke or TIA, widespread screening for, and treatment of, high tHcy in TIA and stroke patients remains experimental and cannot be recommended [46, 47].

**Acknowledgements**

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**Appendix**

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**International Steering Committee:** Clin. Prof. Graeme Hankey (Chairman; Australia), Dr. Christopher Chen (Singapore), Dr. John Gommans (New Zealand), Prof. Kennedy Lees (UK), Dr. Jose Navarro (Philippines), Dr. Udaya Ranawaka (Sri Lanka), Dr. Stefano Ricci (Italy), Dr. Reinhold Schmidt (Austria), Dr. Andrew Slivka (USA) and Dr. Alexander Tsiskaridze (Republic of Georgia).

**Data Monitoring and Safety Committee:** Assoc. Prof. Michael Hobbs (Department of Public Health, University of Western Australia) and Mr. Max Le (biostatistician, Department of Public Health, University of Western Australia).

**Outcome and Adverse Events Adjudication Committee:** Dr. Phil Tuch (consultant neurologist, Fremantle Hospital, Australia), Dr. Paul Langton (consultant cardiologist, Sir Charles Gairdner Hospital, Australia) and Prof. Konrad Jamrozik (Department of Primary Health Care and General Practice, Imperial College, London).

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