VITATOPS STUDY

VITAMINS TO PREVENT STROKE

STUDY

A multi-centre, randomised, double-blind, placebo-controlled, clinical trial examining the efficacy and safety of multi-vitamin therapy in secondary stroke prevention.

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1. Study Summary

There is a large body of consistent, biologically plausible, epidemiological evidence linking hyperhomocysteinaemia (HHC) in a dose-dependent fashion to an increased risk of atherosclerotic vascular morbidity and mortality. Furthermore, HHC is being associated increasingly with an increased risk of vascular dementia, dementia of Alzheimer type, and depression. A simple, non-toxic, and inexpensive therapeutic intervention in the form of multi-vitamins (folate, B_6, B_{12}) has been shown to be highly effective at reducing plasma homocysteine (Hcy) levels, irrespective of the underlying cause. However, the efficacy of Hcy-lowering therapy in reducing vascular injury, and the impact which this may have on vascular morbidity and mortality, dementia and depression remains to be demonstrated.

The VITATOPS study is an international multi-centre, randomised, double-blind, placebo-controlled, clinical trial designed primarily to examine the efficacy and safety of multi-vitamin (folate, B_6, B_{12}) therapy in the prevention of 'stroke, myocardial infarction, or death from any vascular cause', among patients randomised within seven months of a stroke or transient ischaemic attack (TIA) of the eye or brain. Secondary outcomes include TIA, dementia, depression unstable angina and revascularization procedures of the coronary, cerebral and peripheral circulations. It is planned that 8,000 patients will be randomised and followed up for a mean period of 2.5 years (range 1-8 years) by the end of 2009.

However patients were initially invited to consent to randomisation and follow-up for 5 years. These patients who consented for 5 years of participation now have the option to continue on the study post 5 year follow-up or withdraw from the trial (data would then be censored at 5 years for these patients). The trial is designed to detect a relative risk reduction of 15% in the vitamin treated group, with a power of 80% (α=0.05).

2. Background

Stroke remains a substantial burden on stroke patients, their carers and society. It is the third most common cause of death and the most important cause of long term physical disability in developed countries. Each year 0.2% of the population (200 per 100,000) suffer a stroke of whom 65% are dead or disabled at 12 months (1). Despite best medical and surgical therapy (including risk factor modification), hospitalised stroke survivors remain at a significantly increased risk of recurrent vascular events, with an annual risk of stroke, myocardial infarction, and death from any vascular cause, of about 8% (2).

The best strategy for reducing the burden of stroke is prevention, and one approach is to identify and target people at high risk (the 'high risk approach') and treat the underlying cause of stroke. However, for this to be effective, we need to know the cause of stroke, the risk factors or markers of the cause, and have interventions that can favourably modify risk. Not infrequently we cannot identify the underlying cause of stroke; it has been estimated that underlying pathophysiology in more than 40% of strokes remains unexplained (3).

2.1 Hyperhomocysteinaemia is a possible modifiable risk factor for stroke

Risk factors for stroke are characteristics in an individual (or in a population) which indicate that the individual (or population) has an increased risk of the stroke compared with an individual (or population) without those characteristics. This does not necessarily imply that

the risk factor causes the stroke. Rather, the likelihood that any risk factor such as HHC is actually involved in the pathogenesis of stroke, depends on five main factors:

- whether the risk factor is associated independently with stroke and is not just a marker of some other causal risk factor;
- whether the association is consistent across studies, dose-related and strong;
- whether the association between the putative cause and the occurrence of stroke is specific;
- whether the association is biologically plausible; and
- whether reducing the prevalence or level of the risk factor in the population (or group of people in a randomised clinical trial) is followed by a reduction in the incidence of stroke .

In all cases, exposure to the putative causal factor must precede the development of stroke.

2.1.1 Independent, consistent, strong, dose-related association with stroke and other atherosclerotic vascular disease

Stroke

An overview of observational epidemiological studies (including 3 prospective (nested) case-control studies, one prospective cohort study composed of patients with systemic lupus erythematosus, and 12 case-control studies), reveals an independent, linear relationship between increasing levels of Hcy and risk of stroke (4-8*)*. For every 5 μmol/L increment in Hcy level, the risk of stroke or TIA is increased by about 1.5 (95% CI: 1.3 to 1.9) times (4).

For people with fasting plasma homocysteine levels in the upper 10^{th} centile of the population but less than 100 μmol/L the relative risk of stroke or TIA is about 2.5 (95% CI: 1.8 to 2.9) times greater than people with "normal" homocysteine levels (4).

Other atherosclerotic vascular disease

An overview of more than 20 cross-sectional and case-control studies, involving more than 2,500 patients with coronary and peripheral vascular disease demonstrates an independent, strong, graded association between increasing plasma homocysteine levels and atherosclerotic disease of the coronary and peripheral circulation (4-6).

The risk is similar to, and independent of, other risk factors, such as hypercholesterolaemia or smoking, and applies to all categories of vascular disease.

However, the results of the more methodologically sound prospective studies indicate a less strong association between plasma homocysteine and cardiovascular disease in contrast to the less methodologically sound cross-sectional and case-control studies (5,7-9). This suggests that elevated homocysteine levels could be an acute-phase reactant that is predominantly a marker of atherogenesis, or a consequence of other factors more closely linked to risk of cardiovascular disease, rather than a causal risk factor for vascular disease (7).

2.1.2 Biologically plausible

High levels of Hcy have been shown to be both atherogenic and prothrombotic (10-12). *In vitro,* Hcy induces proliferation of smooth muscle cells, causes toxicity to endothelial cells grown in tissue culture by inducing lipid peroxidation, and interferes with the vasodilator and anti-thrombotic functions of nitric oxide (10). *In vivo*, plasma levels of Hcy have been correlated with increasing carotid arterial intimal-medial wall thickness (11), extra-cranial

carotid artery stenosis (12), carotid plaque area (13), and ischaemic stroke due to large and small artery disease and not other aetiological subtypes of stroke (14). Furthermore, endothelial dysfunction, as estimated by increased plasma von Willebrand factor concentrations, is ameliorated after lowering HHC by means of folate and vitamin B_6 treatment (15).

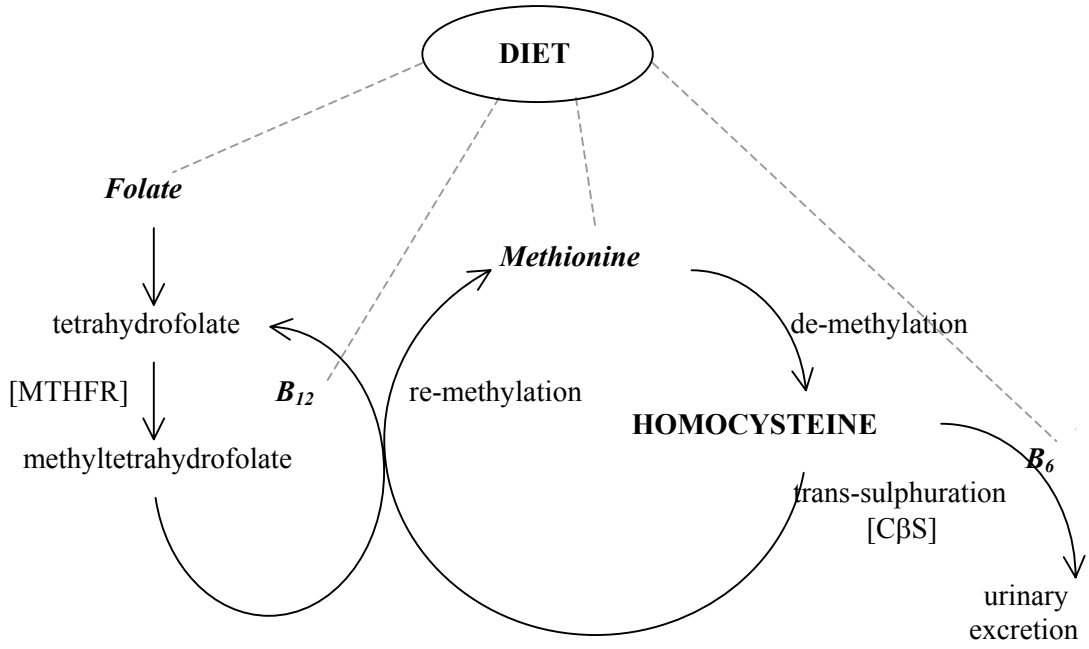

Figure 1: Metabolic pathways of homocysteine metabolism.

MTHFR = methylene tetrahydrofolate reductase
CβS = cystathionine beta-synthase

2.2 Homocysteine metabolism

Homocysteine is derived from the metabolism of the essential sulphur-containing amino acid methionine, which is abundant in widely consumed animal protein. The metabolism of dietary methionine requires at least two principal enzymes and three vitamins.

Methylene tetrahydrofolate reductase (MTHFR) regulates the availability of the methyl group donor 5-methyltetrahydrofolate, which in turn acts as a substrate for methionine synthase to convert homocysteine to methionine, and thus recycle homocysteine. Folate and vitamin B_{12} are cofactors for MTHFR. Cystathione β synthase (CβS) acts with pyridoxine (vitamin B6) as a cofactor for the trans-sulphuration and elimination of Hcy (figure 1).

Dysfunctional enzymes or inadequate amounts of any of these nutrients may therefore lead to an elevated concentration of intracellular Hcy, which is then exported to the plasma, exposing the vascular tissue to the possibly deleterious effects of excess Hcy.

2.3 Factors influencing homocysteine levels

Plasma homocysteine levels are determined by both genetic and nutritional factors.

2.3.1 Genetic factors

Deficiencies in the principal enzymes in the metabolism of homocysteine predispose to elevated plasma homocysteine levels. Severe deficiency of CβS is the most common cause of the classical severe form of homocystinuria (16). At least 17 mutations of the CβS gene have been described in patients with homocystinuria, of which G919A and T833C are the most common (17, 18). The presence of one or more of these mutations is likely to influence homocysteine metabolism, and thus homocysteine levels, but other factors such as vitamin B_6 (pyridoxine) levels are also likely to be relevant in patients with a mutation involving the CβS gene.

Another common cause of hyperhomocysteinaemia is deficiency of MTHFR. The C677T mutation in the gene for MTHFR is common; one in 10 Australians are homozygous for this variant (19). It results in a thermolabile form of the enzyme, and high plasma levels of Hcy among homozygous subjects with low plasma folate (20).

2.3.2 Non-genetic factors

Reduced levels of vitamin co-factors for the key enzymes involved in the re-methylation (folate and vitamin B_{12}) and trans-sulphuration (vitamin B_6) pathways of Hcy metabolism may cause hyperhomocysteinaemia. Other causes of hyperhomocysteinaemia include renal failure (causing impairment of urinary cysteine excretion), various disease states (eg. malignancy) and drugs (eg. Methotrexate) (21). Plasma levels of Hcy increase with age, are slightly higher in males compared with females, and are higher in post-menopausal compared with pre-menopausal women (21).

2.4 Measurement of plasma homocysteine levels

Plasma levels of homocysteine are generally measured whilst the patient is fasting, or after methionine loading. Both are highly interrelated but the former may reflect cobalamin- and folate-dependent remethylation, and the latter, pyridoxal 5'-phosphate-dependent trans-sulfuration (22). Methionine-loading may also expose a latent abnormality of Hcy metabolism in a fashion analogous to the glucose tolerance test (21)

Methionine-loading is more sensitive however, and may expose a latent abnormality of Hcy metabolism in up to 40% of people with a fasting plasma homocysteine level in the "normal" range (6, 23). It is thus somewhat analogous to the glucose tolerance test in diabetes.

Reference ranges for total plasma Hcy vary slightly between laboratories, but most commonly lie within the range of 5 to 15 μmol/l in the fasting state (21). In the setting of an acute occlusive vascular event, levels fall up to 40% during the first 24 hours, corresponding to a reduction in plasma levels of albumin (plasma Hcy is highly bound to albumin) but return towards baseline within 72 hours (24).

2.5 Definition of hyperhomocysteinaemia

An elevated fasting plasma level of Hcy has conventionally been defined as that which exceeds the 90th or 95th centile limits of a normal population. This definition is somewhat arbitrary however, being based on certain "cut-off" points in the normal distribution of plasma levels of homocysteine, in much the same way that high blood pressure and hypercholesterolaemia are defined. It is not based on any particular sudden change in the risk of vascular disease at any of these cut-off points; the relationship between increasing plasma homocysteine levels and increasing risk of vascular disease appears to be linear (5).

There is now evidence however that, in some populations, these "cut-off" points may be too high; for example, as many as 40 to 50% of a recently studied elderly American population were found to consume insufficient folate and B_6 to maintain low plasma concentrations of Hcy (25). Despite recent attempts to define an 'optimal' reference range for plasma Hcy, using a vitamin 'replete' population (26), it remains unclear how to define hyperhomocysteinaemia, particularly in terms of plasma levels that reliably identify patients at increased risk of vascular disease.

2.6 Prevalence of hyperhomocysteinaemia

In spite of a lack of consensus on the definition of hyperhomocysteinaemia, we and others (14, 15) have found that about 40% of patients with first-ever stroke have fasting levels of Hcy in excess of the 95th centile of laboratory controls (27), suggesting that hyperhomocysteinaemia is widely prevalent in stroke patients.

2.7 Homocysteine-lowering therapy

Blood homocysteine levels can be lowered with folic acid and vitamin B_{12} supplementation. A meta-analysis of 12 clinical trials including 1114 individuals with renal failure showed that folic acid at a dose of between 0.5 and 5 mg daily lowers tHcy levels by 25 % (95 % CI: 23-28 %) (28). The minimum effective daily dose of folic acid for achieving maximal homocysteine-lowering efficacy is about 0.5 mg. The effect was more pronounced in subjects with higher tHcy levels and lower folate levels before treatment (28,29). Vitamin B_{12} at a dose of 0.02 - 1 mg further reduces tHcy levels by about 7 % (95 % CI: 3 - 10 %). Vitamin B_6 (2 - 50 mg daily) did not show any additional effect (28). However, vitamin B_6 may be effective in reducing post-methionine loading homocysteine levels and when combined with folate (30-34).

An optimal Hcy-lowering regime would appear to be a combination of folate 2 mg, B_6 25 mg and B_{12} 500 μg daily. Doses of B_6 as low as 200 mg/day run a small risk of causing a neuropathy. However, this side-effect is almost always reported in the setting of chronic use of B_6 (months to years) in daily doses exceeding 1 gram and is reversible (35). As 1 to 3% of oral B_{12} is absorbed by simple diffusion (36), the addition of 500 μg/d of B_{12} will correct the majority of cases of subclinical B_{12} deficiency (Australian RDI for B_{12} = 2 μg/d (29)) and thereby minimise concerns regarding the potential of folate to mask the clinical onset of B_{12} deficiency.

3. Study Objective

3.1 Study Objective

3.1.1 Primary Objective

- To determine whether the addition of vitamin supplements (folate 2 mg, B_6 25 mg, B_{12} 500 μg) to best medical / surgical management (including modification of risk factors) will reduce the combined incidence of recurrent vascular events (stroke, myocardial infarction) and vascular death in patients with recent stroke or transient ischaemic attack (TIA)

3.1.2 Secondary Objectives

- To determine whether the addition of vitamin supplements (folate 2 mg, B_6 25 mg, B_{12} 500 μg) will reduce the incidence of dementia and depression in patients with recent stroke or TIA.
- To determine whether the addition of vitamin supplements (folate 2 mg, B_6 25 mg, B_{12} 500 μg) will reduce the occurrence of TIA in patients with recent stroke or TIA.
- To determine whether the addition of vitamin supplements (folate 2 mg, B_6 25 mg, B_{12} 500 μg) will reduce the incidence of peripheral arterial disease (PAD) as measured by leg amputation in patients with recent stroke or TIA.
- To determine whether the addition of vitamin supplements (folate 2 mg, B_6 25 mg, B_{12} 500 μg) will reduce the incidence of the primary outcome event (stroke, MI or vascular death) in patient subgroups such as those of different ethnicity and genotype.

4. Study Design

The VITATOPS study is a multi-centre, randomised, double-blind, placebo-controlled trial over nine years (2000-2008).

Randomisation codes are generated and stratified by hospital. Randomisation will be by means of a central 24-hour telephone service or via an interactive website (http://vitatops.highway1.com.au), which will also allow a final check of eligibility prior to randomisation. After baseline data have been provided by the investigator, the randomisation service allocates the patient a randomisation treatment pack number which is to be recorded on the Enrolment form.

5. Patients

5.1 Inclusion criteria

All patients presenting within seven months of stroke (ischaemic or haemorrhagic) or TIA (eye or brain) are eligible for this trial.
In addition, the patient must:

- agree to take study medications
- be geographically accessible for follow-up

- provide written informed consent

5.1.1 Criteria for the diagnosis of stroke

An acute new disturbance of focal neurological function lasting more than 24 hours, and thought to be due to focal brain infarction, or non-traumatic, non-neoplastic intracerebral haemorrhage or subarachnoid haemorrhage.

5.1.2 Criteria for the diagnosis of TIA of the brain or eye

An acute new disturbance of focal neurological or monocular function with symptoms lasting less than 24 hours and thought to be due to brain or ocular ischaemia.

5.2 Exclusion criteria

5.2.1 General

- taking folic acid or B_6 on medical advice
- use of vitamin supplements containing folate, B_6 or B_{12} (unless patient agrees to take study medication instead of the vitamin supplements which they usually take)
- taking Methotrexate for any reason
- pregnancy or women of child-bearing potential who are at risk of pregnancy
- limited life expectancy

5.3 Baseline data

Table 1: Data Collection Chart

	Enrolment and Randomisation	1-3 months	6 months	12, 24 ,36, 48, 60 months	18, 30, 42, 54 months
Consent	✓				
Clinical history & examination	✓			if indicated	if indicated
Enrolment Form and Baseline Data	✓				
CT scan	if available				
Carotid Doppler studies	if available				
Creatinine	if available		if available	if available	
Fasting Glucose and Lipids	if available		if available	if available	
Follow-up / Adverse Events		✓	✓	✓	✓
HAD Scale			☆	☆	
MMSE			☆	☆	

✓ = compulsory
☆ = optional
HAD Scale: Hospital Anxiety and Depression Scale
MMSE: Mini Mental State Examination

5.3.1 Cholesterol, creatinine, triglycerides, glucose, carotid doppler and cerebral imaging studies

These investigations are not required by trial protocol. However, if results are available, they should be recorded on the baseline data sheet.

5.3.2 Dementia and depression scores

Investigators are required to record any history of treatment for depression on the Baseline Form. HAD Scale and MMSE Forms will be used as assessment tools for dementia and depression, respectively. Completion of MMSE and HAD Scale Forms are optional.

6. Intervention

6.1 Trial medication

- Active treatment arm: folate 2 mg, B_6 25 mg and B_{12} 500 μg daily as a single tablet.
- Placebo arm: The placebo tablet will have the same appearance, taste and texture as the vitamin preparation and contains excipients, coating and coating aids.

After randomisation, the patient is to be given the allocated numbered Randomisation pack, containing either vitamin supplements (folate 2 mg, B_6 25 mg, B_{12} 500 μg) or matching placebo.

Treatment should commence as soon as is possible after randomisation, and ideally, this will correspond to the time of commencement of other routine secondary prevention strategies such as Aspirin. Medications should be administered either orally or in crushed form via a nasogastric feeding tube.

Subsequent supplies of trial medication are to be issued at the time of the six-monthly follow-up appointments.

6.2 Cessation of trial medication

Trial medication may be discontinued at the patient's request or in the setting of a serious adverse event (see section 8). All these patients should, however, continue to be followed up for outcome events or death from any cause.

Temporary interruption of the trial medication, for example because of surgery, is permitted, but should be as brief as possible. This should be reported at the next follow-up visit.

7. Follow-Up Visits

Follow-up visits every six months are requested as part of the VITATOPS study until completion of the trial. A follow-up phone call is also requested one to three months after recruitment to the trial.

Initially, follow-up visits were requested every six months until 5 years participation.

At each follow-up visit or phone call, a Follow-up Data Form should be completed. Data required include: outcome events, secondary stroke prevention measures (smoking, blood pressure, anti-hypertensive therapy, aspirin therapy, lipid-lowering therapy, anti-diabetic medications, anticoagulation), cardiovascular surgery (carotid, coronary, aortic, peripheral vascular) or angioplasty, treatment for depression or diagnosis of dementia since the last follow-up appointment, occurrence of major events (outcome events or death from any cause), adverse events, use of vitamins and compliance with trial medication (questioning, tablet count, return of tablet container).

Completion of HAD Scale and MMSE Forms is optional. However, if HAD Scale and MMSE Forms are completed at 6 month follow-up, subsequent HAD Scale and MMSE Forms are required to be completed at requested follow-up visits or phone calls.

Patients should continue to have regular follow-up appointments as per appointment schedule even after the occurrence of an outcome event. Similarly, if at all possible, follow-up should continue after premature termination of the study medication, and should be maintained until completion of the study.

To avoid patients being lost to follow-up, the patient's general practitioner can be informed about the VITATOPS study by means of a special letter.

If a patient does not keep a follow-up appointment, he or she may have experienced an outcome event. It is therefore vitally important that additional information about the patient is sought in this situation. No patient should be lost to follow-up.

8. Outcome Measures

The primary outcome event is the composite event 'stroke, myocardial infarction, or death from any vascular cause', whichever occurs first. If the cause of death is unknown, this shall be recorded as 'presumed vascular death – cause unknown'.

Secondary outcome measures include non vascular death, TIA, depression, dementia, unstable angina, revascularization procedures of the coronary, cerebral and peripheral circulations.

An independent, blinded Auditing committee will audit every outcome event and additional relevant information may be sought from the participating neurologist or physician-in-charge. Individual members of the blinded Auditing Committee will classify the event, and if there is disagreement, the event will be reviewed by the Steering Committee who will make a final decision. The final classification will be included in the study database.

8.1 Death from vascular causes

Death due to stroke, myocardial infarction, sudden presumed cardiac death, cardiac failure, ruptured aortic aneurysm or pulmonary embolism.

Vascular causes of death

- Fatal ischaemic stroke: death within 28 days of ischaemic stroke due to the effects of the stroke. These include direct neurological effects of the stroke and indirect fatal complications of the stroke.
- Fatal haemorrhagic stroke: death within 28 days of haemorrhagic stroke due to the direct or indirect effects of the stroke.
- Fatal stroke of unknown pathological type.
- Fatal myocardial infarction: death within 28 days of a documented myocardial infarction and due to the direct or indirect effects of the myocardial infarction.
- Definite sudden death: witnessed sudden death with reliable observation of timing, ie. patient died within one hour of onset of symptoms.

- Probable sudden death: witness available but unreliable data on timing of events, or found dead and previously 'healthy'.
- Cardiac failure: death due to cardiac failure.
- Fatal ruptured aortic aneurysm or aortic dissection: death within 28 days of the ruptured aorta.
- Fatal pulmonary embolism: death within 28 days pulmonary embolism (confirmed by ventilation perfusion scanning or pulmonary angiography) and due to the direct or indirect effect of the pulmonary embolus.

8.2 Criteria for the diagnosis of stroke

An acute new disturbance of focal neurological function resulting in death or lasting more than 24 hours, and thought to be due to brain infarction or intracranial haemorrhage.

8.2.1. Ischaemic stroke

A clinically definite stroke in which CT or MRI brain scan, performed within three weeks of onset of symptoms, was either normal or showed an infarct in the clinically relevant area of the brain; or autopsy showed evidence of relevant brain infarction.

Subtypes of ischaemic stroke

Large artery cerebral infarction

Ischaemic stroke with:

(a) clinical, duplex ultrasound, MRI angiographic or angiographic evidence of disease of the extracranial or intracranial large artery supplying the ischaemic area of the brain, including the aortic arch, common and internal carotid artery, main stem of the middle cerebral artery, vertebral artery, and basilar artery, *and*
(b) no major cardioembolic source (see below), *and*
(c) clinical opinion that the most likely cause of the brain infarct was large artery disease causing in-situ thrombosis or thrombo-embolism.

Small artery cerebral infarction

Ischaemic stroke with:

(a) retained consciousness and higher cerebral function; *and*
(b) CT or MRI brain scan that was normal or showed a small infarct in the basal ganglia, internal capsule, or brainstem; *and* either
(c) one of the classical lacunar syndromes *(lacunar infarct)*
    - pure motor hemiparesis,
    - pure hemisensory loss,
    - pure hemisensori-motor loss,
    - ataxic hemiparesis

or

(d) a non-lacunar small artery syndrome *(non-classical lacunar infarct)*

e.g. basilar branch artery syndromes

Cardioembolic cerebral infarction
Ischaemic stroke with:
(a) a major cardioembolic source (e.g. atrial fibrillation, myocardial infarction in the preceding 6 weeks or echocardiographic evidence of left ventricular thrombus, endocarditis, or a prosthetic heart valve), *and*
(b) no definite evidence of large artery disease (see above), *and*
(c) clinical opinion that the most likely cause of the brain infarct was embolism from the heart.

Cerebral infarction of unknown or uncertain cause
Definite ischaemic stroke which does not meet the above criteria, or where there is more than one possible explanation (e.g. a patient with atrial fibrillation and carotid stenosis).

Retinal infarction
An acute painless and persistent (beyond 24 hours) monocular loss of visual acuity or visual field with ophthalmoscopic findings of pallor of the retina. Additional findings often included an afferent pupillary defect, embolic material in the retinal arteries or arterioles, or a cherry red spot over the fovea in cases of central retinal artery occlusion.

8.2.2 Haemorrhagic stroke

Intracerebral haemorrhage
A clinically definite stroke in which brain CT, MRI or autopsy show evidence of haemorrhage into the clinically relevant area of the brain, excluding haemorrhagic transformation of infarction, haemorrhage into a tumour, and haemorrhage secondary to trauma.

Subarachnoid haemorrhage
Typical clinical syndrome of sudden onset of headache; with or without neck stiffness and focal neurological signs; and with CT, cerebrospinal fluid, or autopsy evidence of bleeding primarily in the subarachnoid space.

8.2.3 Stroke of unknown pathological type

A clinically definite stroke not documented by CT or MRI brain scan, or autopsy.

8.2.4 Procedure-related stroke

Stroke occurring within seven days of a medical or surgical intervention which is judged to be relevant to the aetiology of the stroke.

8.2.5 Transient ischaemic attack of the brain or eye

Transient ischaemic attack (TIA) is a secondary outcome event in the VITATOPS study. A definition has been provided here to distinguish TIA from stroke – which is a primary outcome event.

An acute new disturbance of focal neurological or monocular function with symptoms lasting less than 24 hours and thought to be due to brain or ocular ischaemia.

8.3 Myocardial infarction

At least two of the following characteristics have to be documented:

- a history of chest discomfort for at least 20 minutes or terminated by collapse or opioid medication.
- serum cardiac enzymes (CK, CK-MB) or serum troponin-I or troponin-T more than twice the upper limit of normal. In certain circumstances which report abnormal results rather than abnormal range, we will accept what is classified as abnormal locally.
- the development of specific abnormalities (e.g. Q-waves) on the standard 12-lead ECG.

8.4 Dementia and depression

The MMSE & HAD Scale will be used as assessment tools for dementia and depression. Depression and anxiety will be reported where patients score eight or more for both the anxiety and depression questions for the HAD Scale (37). Dementia will be diagnosed where patients score less than 23/30 for MMSE (38).

9. Adverse Events

The main potential risks to patients are:

- Masking of the development of B_{12} deficiency by folate supplementation. This is an extremely rare clinical event, and the risk of this happening will be even further minimised by the addition of B_{12} to the study medication.
- B_6 neuropathy. There have been isolated reports of neuropathy with doses as low as 200 mg/day. This reversible side-effect is however, almost always reported in the setting of chronic use of B_6 (months to years) in daily doses exceeding 1g, and there have been long-term studies using doses of 100 to 150 mg/d without significant toxicity or adverse effects (35).

If there is a clinical suspicion of the development of neuropathy, assays of vitamin B_6 and B_{12} are recommended. The patient should otherwise be managed according to the discretion of the responsible investigator. If the study medication is implicated in the development of neuropathy, it should be ceased.

- Others. All collaborating centres are to record other major adverse event as follows:
  - any potentially fatal or life threatening event,
  - any permanently or severely disabling event,
  - any event that requires hospitalisation (or if study participant is an in-patient any event that prolongs hospitalisation),
  - any cancer, or
  - any other comparable medical event requiring significant therapeutic intervention.

The unblinded Data Monitoring and Safety Committee will review all adverse events and report to the Steering Committee.

10. Statistical Aspects

10.1 Sample size

The expected annual incidence of the primary outcome event (stroke, myocardial infarction or vascular death) is 8% (2).

Table 2 gives sample sizes required to detect a 10%, 15% and 20% reduction in the expected 8% annual incidence of the primary outcome event, based on follow-up of one, two or three years, for 80% power and for 90% power. The numbers in the body of each table are those for *each* study group and foreshadow a trial of twice the size indicated, assuming no cross-over or losses to follow-up. Calculations are based on analysis of a 2x2 table using χ^2 with Yates correction.

Our sample size analysis has been based upon an expected 8% annual incidence of the primary outcome event in the placebo group, accrual of patients over a 4 year period, intervention and placebo groups of equal size, a minimum follow-up of one year for the last patient randomised, a type I error of 5% and type II error of 20%. Assuming an average follow-up of 2 5 years a sample size of 3,982 patients would be required in each group. We have set our target sample size at 8,000 patients.

10.2 Interim analyses

The data safety and monitoring committee (DSMC) will conduct interim analyses after follow-up of 5,000 and 10,000 person years. Using statistical criteria for acceptable deviations from the null hypothesis, the committee will advise the investigators whether the recruitment can continue or whether the study should be terminated. The DSMC will advise the steering committee if adverse events in each treatment group differ by more than 2.58 standard deviations ($p<0.01$) and if the primary outcome event rate in each treatment group differs by more than 3.29 standard deviations ($p<0.001$) and suggest that, if this case arises, the trial code be broken with a view to terminating recruitment.

Table 2: Size of *each* group for follow-up of one, two or three years

Follow-up	1 year			2 years			3 years		
Risk in untreated group (%)	8	8	8	8	8	8	8	8	8
Risk in treated group (%)	7.2	6.8	6.4	7.2	6.8	6.4	7.2	6.8	6.4
Relative effect (%)	10	15	20	10	15	20	10	15	20
80% power	17,475	7,636	4,221	9,081	**3,982**	2,193	6,317	2,751	1,523
90% power	23,314	10,168	5,609	12,113	5,300	2,913	8,424	3,661	2,023

10.3 Final analysis

The final analysis will compare the incidence of the primary outcome event between the vitamin and placebo treatment groups over the duration of follow-up. Two analytical strategies will be employed: an intention-to-treat and on-treatment analysis. The primary analysis will be an intention-to-treat analysis. Events in the on-treatment analysis will only be included 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 stroke, myocardial infarction, or vascular death, 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 identify subgroups of patients at highest risk of recurrent vascular events, and those who achieve maximal benefit from Hcy-lowering therapy, the following subgroup analyses are planned (with the primary study outcome as the dependent variable): age, sex, ethnicity, clinical syndrome, pathology (TIA, ischaemic stroke, haemorrhagic stroke), pathogenesis (large vessel, small vessel, embolic, primary intracerebral haemorrhage), stroke severity (Oxford Handicap Score ≤2, ≥3) and MTHFR genotype.

11. Ethical Aspects

11.1 Declaration of Helsinki

This trial will be performed in agreement with the Declaration of Helsinki.

11.2 Informed consent

After verification of the inclusion and exclusion criteria, patients will be asked to provide written informed consent according to the requirements of the Local Institutional Ethics Review Committee at each participating centre. A Sample Patient Information Brochure, Patient Consent Form is attached.

Initially, patients were invited to consent at randmomisation to follow-up for 5 years. Patients now have the option to continue follow-up until completion of the study or withdraw (considered 'censored') at the 5 year time-point.

12. Study Folders

A study folder will be kept for each trial patient. This will contain:

- Patient information brochure and consent forms
- Copy of inclusion and exclusion criteria
- Enrolment Form
- Baseline Data Form
- Follow-up Data Form
- Major Events Form
- MMSE Form
- HAD Scale Form

13. Publication of Trial Results

Publication of the trial results will be on behalf of all the participating centres, and in the name of the "VITATOPS Study Group". Before submission of the main results paper, all investigators will have the opportunity to comment. All subsequent papers will be published in the name of the "VITATOPS Study Group" followed by: participating country (alphabetical order), institution/collaborating centre (alphabetical order), local chief clinician and finally all other clinicians who recruited patients (alphabetical order). Investigators may publish on patients in their own centres after the main results have been published.

14. Trial Organisation

Web site:http://vitatops.highway1.com.au

14.1 Trial Office

Stroke Unit
Royal Perth Hospital
PO Box X 2213
Perth, Western Australia, 6847
Phone (+61) (8) 9224 7004
Fax (+61) (8) 9224 8424

14.2 Data and Biostatistical Service

University Department of Public Health
University of Western Australia
Stirling Highway, Nedlands, 6009
Western Australia
Phone (+61) (8) 9380 1260
Fax (+61) (8) 9380 1188

14.3 Central Laboratory

Department of Core Clinical Pathology and Biochemistry
Royal Perth Hospital
Perth, Western Australia, 6847
Phone (+61) (8) 9224 2897
Fax (+61) (8) 9224 3449.

14.4 Tablet Distribution Centre

Department of Pharmacy
Royal Perth Hospital
Wellington Street,
Perth, Australia, 6847
Phone (+61) (8) 9224 1455
or (+61) (8) 9224 2478
Fax (+61) (8) 9224 2939

14.5 Investigators

To promote clear and uniform communication between the participating centre and the Trial Office, it is preferred that there will be a single neurologist or general physician at each centre who is responsible for the organisation of the VITATOPS study in his/her centre.

14.6 Steering Committee

Chairman:

Clinical Prof GJ Hankey	Head, Stroke Unit, Royal Perth Hospital

Members include (in alphabetical order):

Assoc Prof RI Baker	Haematologist, Royal Perth Hospital
Dr J Eikelboom	Assoc Prof, Department of Medicine, McMaster University
Ms A Gelavis	New Business Co-ordinator, ORION Laboratories
Dr SC Hickling	NHFA Research Fellow, School of Population Health, University of Western Australia
Prof K Jamrozik	Professor, School of Population Health University of Queensland
Assoc Prof FM van Bockxmeer	School of Surgery & Pathology, University of Western Australia and Department of Core Clinical Pathology and Biochemistry, Royal Perth Hospital
Dr S Vasikaran	*Ex Officio* Head, Department of Core Clinical Pathology & Biochemistry, Royal Perth Hospital

The Steering Committee carries the responsibility for the trial. Tasks include:

- Approval of the study protocol and any amendments.
- Prevention, recognition and resolution of problems that may interfere with the conduct of the trial.
- Classification of outcome events on which no consensus is reached by the Auditing Committee.
- Deciding whether or not the trial should continue, based on the recommendations of the Data Monitoring and Safety Committee.
- Writing manuscripts.

14.7 International Steering Committee

Members include (in alphabetical order):
Professor Ale Algra (Netherlands)
Dr Christopher Chen (Singapore)
Dr Raymond Cheung / Dr Lawrence Wong (Hong Kong)
Dr Ivana Divjak (Serbia)
Professor José Ferro (Portugal)
Dr Gabriel R. de Freitas (Brazil)

Dr John Gommans (New Zealand)
Professor Stanislav Groppa (Moldova)
Clin. Prof. Graeme Hankey (Australia)
Assistant Professor Michael D. Hill / Professor J. David Spence (Canada)
Professor Kennedy Lees (United Kingdom)
Professor Liu Lisheng (China)
Dr Jose C. Navarro (Philippines)
Dr Udaya Ranawaka (Sri Lanka)
Dr Stefano Ricci (Italy)
Associate Professor Reinhold Schmidt (Austria)
Dr Andrew Slivka (United States)
Dr Kay-Sin Tan (Malaysia)
Associate Professor Alexander Tsiskaridze (Republic of Georgia)
Dr Wasim Uddin (Pakistan)
Dr Geert Vanhooren (Belgium)
Dr Denis Xavier (India)

Others:……..

14.8 Data Monitoring and Safety Committee

Tasks include:

- Analyses of unblinded interim data.
- Unblinded analysis of adverse events.
- Formulation of recommendations to the Steering Committee on the continuation of the trial.
- Offering unsolicited recommendations to the Steering Committee, for example after publication of results of a similar trial.

Members:

Dr Jane Armitage	*Reader in Clinical Epidemiology & Honorary Consultant in Public Health Medicine*, Clinical Trials Service Unit & Epidemiological Studies Unit, Radcliffe Infirmary, Oxford, UK
	Dr Michael Hobbs *Ex Officio* Associate Professor, Department of Public Health, University of Western Australia
Mr Max Le	*Ex Officio* Biostatistician, Department of Public Health, University of Western Australia
Dr Cathie Sudlow	*Clinical Senior Lecturer, Wellcome Trust Clinician Scientist and Honorary Consultant Neurologist,* Division of Clinical Neurosciences, University of Edinburgh, Western General Hospital, UK
Prof Keith Wheatley	*Professor of Medical Statistics*

	Co-Director, University of Birmingham Clinical Trials Unit, UK
Associate Professor Qilong Yi	*Senior Biostatistician* Canadian Blood Services National Epidemiology and Surveillance *Associate Professor* Public Health Science University of Toronto

14.9 Auditing Committee

Tasks include:

- blinded classification of outcome events

Members:

Dr William Brown	School of Medicine, Health Policy & Practice. University of East Anglia
Dr Marcel Bulder	Department of Neurology, UMC, Utrecht
Dr John Eikelboom	Haematology Research Fellow, Royal Perth Hospital
Clinical Professor GJ Hankey	Head, Stroke Unit, Royal Perth Hospital
Dr Wai Khoon Ho	Clinical Researcher, School of Medicine & Pharmacology, Royal Perth Hospital
Professor Konrad Jamrozik	Professor, Department of Public Health, University of Western Australia
Dr Karin Klijn	Registrar, Royal Perth Hospital
Dr Esther Koedam	University of Utrecht
Dr Paul Langton	Consultant Cardiologist, Sir Charles Gairdner Hospital

14.10 Finances

The VITATOPS Trial Office has received funding from the National Health and Medical Research Council of Australia, the National Heart Foundation of Australia and the Health Department of Western Australia. Vitamin tablets and matching placebo tablets for the VITATOPS Study have been supplied by Blackmores Ltd., 23 Roseberry St, Balgowlah, NSW, 2093.

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16. Appendices

How to enrol patients in the VITATOPS study

1. Identify eligible patients

⬇

Inclusion criteria

All patients presenting to one of the participating neurologists or general physicians within seven months of stroke (ischaemic or haemorrhagic) or TIA (eye or brain) are eligible for this trial (see section 4 of protocol for definitions) In addition, the patient must:

- *agree to take study medications*
- *be geographically accessible for follow-up*
- *provide written informed consent*

Exclusion criteria

- *taking folic acid or B_6 on medical advice*
- *use of vitamin supplements containing B_6, B_{12} or folate (unless patient agrees to take study medication instead of the vitamin supplements which they usually take)*
- *taking Methotrexate for any reason*
- *pregnancy or women of child-bearing potential who are at risk of pregnancy*
- *limited life expectancy*

⬇

2. 2. Obtain written consent from the patient for the VITATOPS study

⬇

3. Complete the Enrolment Form *(back of this sheet)* and ring FreeCall XXX XXXX for VITATOPS randomisation number

Or **go to the VITATOPS randomisation website: http://vitatops.highway1.com.au/**

⬇

4. Complete the Baseline Data Form *(gold sheet)*

⬇

5. Write a prescription for 6 months supply of VITATOPS tablets with VITATOPS randomisation number or dispense VITATOPS tablets with the correct VITATOPS randomisation number

⬇

6. Make a follow-up appointment for 1-3 months from randomisation date

⬇

7. Fax the Baseline Data and Randomisation Form to FreeFax XXX XXX

Patient name & contact details:

Name _____ Medical record number _____ Phone _____

Date of birth _____/_____/_____ Gender: Male (1) Female (2)

Name / phone / address of patient's GP _____

Name and phone number of friend or relative not living with the patient _____

VITATOPS Enrolment Form

Please use a black pen

Centre details:

Collaborating centre _____ Randomising Dr/RN _____ Signature_____

Patient details:

Patient Initials _____ Medical record number _____ Date of birth _____/_____/_____

Gender: Male (1) Female (2)

Details of Primary Event *(please circle):*

Date of event		_____/_____/_____	
Monocular blindness?		Yes (1)	No (2)
Dysphasia / Aphasia?		Yes (1)	No (2)
Hemianopia?		Yes (1)	No (2)
Sensory neglect?		Yes (1)	No (2)
Paralysis:	a. of Face?	Yes (1)	No (2)
	b. of Arm?	Yes (1)	No (2)
	c. of Leg?	Yes (1)	No (2)
Sensory disturbance?		Yes (1)	No (2)
Brainstem or cerebellar involvement?		Yes (1)	No (2)

Other impairments? *(please specify)* _____

Cerebrovascular Pathology *(please circle):*

1) TIA of the eye or brain
2) Primary intracerebral haemorrhage
3) Cerebral infarction (cerebrum, brainstem, cerebellum)
4) Uncertain
5) Subarachnoid haemorrhage
6) Retinal infarction

Evidence for Pathology *(please circle):*

1) Clinical only
2) Clinical plus CT or MRI (eg. confirms/excludes haemorrhage)
3) Other *(please specify)* _____

Cerebrovascular Aetiology *(please circle):*

1) Large artery disease
2) Small vessel disease
3) Embolism from the heart
4) Stroke or TIA of unknown or uncertain cause
5) Not Applicable (if CV Pathology is primary intracerebral haemorrhage or subarachnoid haemorrhage)

Current Medications:

Anti-platelet drugs (eg aspirin, clopidogrel, dipyridamole)	Yes (1)	No (2)
Anticoagulants (eg warfarin)	Yes (1)	No (2)
Antidepressants (eg effexor, zoloft)	Yes (1)	No (2)

Please specify any other medications _____

_____

Vitamins (oral or injections) Yes (1) No (2)

(please specify any vitamins) _____

Please phone XXX XXX to RANDOMISE your patient (remember to have your centre access code ready)
***Or* go to the VITATOPS on-line randomisation website: http://vitatops.highway1.com.au**

Date of randomisation ___/___/____ VITATOPS number _____

Please write a prescription for 6 months supply of VITATOPS tablets with the VITATOPS randomisation number
Please FAX this form now to: VITATOPS Trial coordinator Fax: XXX XXX
Please complete the gold Baseline Data Form on day of randomisation

VITATOPS Baseline Data Form

Please use a black pen

Centre and Patient Details:

Patient initials _____ Randomising Dr/RN _____ VITATOPS number _____

Past Medical History

Stroke (including randomising event)?	Yes (1)	No (2)	If yes, date of first ever stroke ___/___/____
Myocardial infarction?	Yes (1)	No (2)	
Ischaemic limb?	Yes (1)	No (2)	

Past Surgical History

Carotid endarterectomy or angioplasty /stent ?	Yes (1)	No (2)
Coronary artery bypass graft or angioplasty / stent?	Yes (1)	No (2)
Aorto-femoral-popliteal bypass graft or angioplasty / stent?	Yes (1)	No (2)

Risk Factors

Hypertension:	- history of (previous or current) ?	Yes (1)	No (2)
	- treated at the time of the event?	Yes (1)	No (2)
	- current blood pressure *(systolic/diastolic)*	_____ /_____	*(seated, right arm, Phase V Korotkov sound)*
Smoking:	- ever?	Yes (1)	No (2)
	- current / at the time of the event?	Yes (1)	No (2)
Hypercholesterolaemia (cholesterol ≥ 6.5 mmol/L)	- history of?	Yes (1)	No (2) Unsure (3)
	- treated at the time of the event?	Yes (1)	No (2)
Diabetes mellitus?		Yes (1)	No (2)
Ischaemic heart disease	-history of (previous or current)?	Yes (1)	No (2)
Atrial fibrillation:	-history of (previous or current)?	Yes (1)	No (2)
Peripheral vascular disease (intermittent claudication)	- history of ?	Yes (1)	No (2)
Depression	- history of (previous or current)?	Yes (1)	No (2)
Alcohol:	- intake at the time of the event?	_____ drinks/day *(1 drink =10g alcohol)*	
Ethnic Background:	- country of birth	_____	
	- describe participants, parents and grandparents ethnic background *(see back of this sheet for categories)*		

_____

Oxford Handicap Score at Time of Randomisation (0) (1) (2) (3) (4) (5) *(see back of this sheet for categories)*

Blood Test Results (if available)

Homocysteine (fasting)	_____	(μmol/L)
Post ML homocysteine	_____	(μmol/L)
Cholesterol (fasting)	_____	(mmol/L)
Triglycerides (fasting)	_____	(mmol/L)
Creatinine	_____	(μmol/L)
Glucose (fasting)	_____	(mmol/L)
Vitamin B12 (Cyanocobalamin)	_____	(pmol/L)

DNA sample collected and stored ? (optional)	Yes (1)	No (2)
HISS sub-study samples collected ? (RPH only)	Yes (1)	No (2)

1. **Please inform the patient's GP of their participation in VITATOPS**
2. **Please make a follow up appointment for 1-3 months from now**

Ethnic Background Categories

- **Caucasian**
- **African**
- **Oriental** (east of Urals to Japan and Philippines; south to Black Sea)
- **South Asian** (south of Himalayas)
- **Middle East** (from Mediterranean to Afghanistan)
- **Oceania** (Melanesian, Polynesian (includes Maori and Pacific Islanders), Micronesian)
- **Other indigenous** (Aboriginal, Torres Strait Islander, Sami, Inuit, Native American, etc.)

Oxford Handicap Score

0) No symptoms
1) Minor symptoms that do not interfere with lifestyle
2) Minor handicap, symptoms that lead to some restriction in lifestyle but do not interfere with the patient's capacity look after himself / herself
3) Moderate handicap, symptoms that significantly restrict lifestyle and prevent totally independent existence
4) Moderately severe handicap, symptoms that clearly prevent independent existence though not needing constant attention
5) Severe handicap, totally dependent patient requiring constant attention night and day

VITATOPS Follow-up Data Form (1-3 months)

Please use a black pen

Centre and Patient Details:

Date of Randomisation: ___/___/___

Patient initials _____ Randomising Dr/RN _____ VITATOPS number _____ Today's date ___/____/_____

Primary & Secondary Outcome Events or Adverse Event *(since last follow-up)*: Yes (1) No (2)

If Yes, please fill out a blue Major Events Form

Vascular Surgery or Angioplasty / Stent *(since last follow-up)*: Yes (1) No (2)

If Yes, specify type _____

Current Risk Factors *(if deceased the following applies to the period immediately prior to patient's death)*

Current blood pressure *(systolic/diastolic)* _____ /_____ *(seated, right arm, Phase V Korotkov sound)*

Current smoker ? Yes (1) No (2)

Atrial fibrillation ? Yes (1) No (2)

Ischaemic heart disease ? Yes (1) No (2)

Peripheral vascular disease ? Yes (1) No (2)

Current Medications:

Anti-platelet drugs ? Yes (1) No (2)

Anti-coagulants ? Yes (1) No (2)

Anti-hypertensives ? Yes (1) No (2)

Lipid- lowering therapy ? Yes (1) No (2)

Treatment (diet or other) for diabetes mellitus ? Yes (1) No (2)

Anti-depressants ? Yes (1) No (2)

Please specify any other medication _____

_____

VITATOPS Medication Compliance *(Collect returned bottle and label with the patient name, today's date and VITATOPS number)*

On average, since the last follow up how many times per week was the trial medication taken?

(0) times per week
(1-2) times per week
(3-4) times per week
(5-6) times per week
(7) times per week

Did patient **stop** the trial medication at all since the last follow up? Yes, temporarily (1) Yes, permanently (2) No (3)

If Yes, specify dates and reason _____

Is the patient taking vitamins **other than** the trial medication Yes (1) No (2)

If Yes, please specify: _____

Has the patient been treated for depression since last review? Yes (1) No (2)

Has the patient been diagnosed with dementia since last review? Yes (1) No (2)

Blood Test Results (if available)

Homocysteine (fasting) _____ (µmol/L)

Cholesterol (fasting) _____ (mmol/L)

Triglycerides (fasting) _____ (mmol/L)

Creatinine _____ (µmol/L)

Glucose (fasting) _____ (mmol/L)

Please make a follow-up appointment for 3-5 months from now (ie 6 months post randomisation)

Please FAX this form now to: VITATOPS Trial coordinator Fax: XXX XXX

VITATOPS Follow-up Data Form

Please use a black pen

Centre and Patient Details:

Date of Randomisation: ___/___/___

Patient initials _____ Randomising Dr/RN _____ VITATOPS number _____ Today's date ___/____/_____

Primary & Secondary Outcome Events or Adverse Event *(since last follow-up)***:** Yes (1) No (2)

If Yes, please fill out a blue Major Events Form

Vascular Surgery or Angioplasty / Stent *(since last follow-up)***:** Yes (1) No (2)

If Yes, specify type _____

Current Risk Factors *(if deceased the following applies to the period immediately prior to patient's death)*

Current blood pressure *(systolic/diastolic)* _____ /_____ *(seated, right arm, Phase V Korotkov sound)*

Current smoker ? Yes (1) No (2)

Atrial fibrillation ? Yes (1) No (2)

Ischaemic heart disease ? Yes (1) No (2)

Peripheral vascular disease ? Yes (1) No (2)

Current Medications:

Anti-platelet drugs ? Yes (1) No (2)

Anti-coagulants ? Yes (1) No (2)

Anti-hypertensives ? Yes (1) No (2)

Lipid- lowering therapy ? Yes (1) No (2)

Treatment (diet or other) for diabetes mellitus ? Yes (1) No (2)

Anti-depressants ? Yes (1) No (2)

Please specify any other medications _____

_____

VITATOPS Medication Compliance *(Collect returned bottle and label with the patient name, today's date and VITATOPS number)*

On average, since the last follow up how many times per week was the trial medication taken?

(0) times per week
(1-2) times per week
(3-4) times per week
(5-6) times per week
(7) times per week

Did patient **stop** the trial medication at all since the last follow up? Yes, temporarily (1) Yes, permanently (2) No (3)

If Yes, specify dates and reason _____

Is the patient taking vitamins **other than** the trial medication Yes (1) No (2)

If Yes, please specify: _____

Has the patient been treated for depression since last review? Yes (1) No (2)

Has the patient been diagnosed with dementia since last review? Yes (1) No (2)

Dementia and Depression (optional)

MMSE score _____ (please also fax a copy of the MMSE test sheet to the trial office)

HAD Score A _____ D _____ (please also fax a copy of the HADS test sheet to the trial office)

Blood Test Results (if available)

Homocysteine (fasting) _____ (µmol/L)

Cholesterol (fasting) _____ (mmol/L)

Triglycerides (fasting) _____ (mmol/L)

Creatinine _____ (µmol/L)

Glucose (fasting) _____ (mmol/L)

Please write a prescription for 6 months supply of VITATOPS tablets with the VITATOPS randomisation number and make a follow-up appointment for 6 months from now

Please FAX this form now to: VITATOPS Trial coordinator Fax: XXX XXX

VITATOPS Major Events Form *(page 1)*

Please use a black pen

Patient name: _____ Signature of randomising Dr/RN:_____ VITATOPS number: _____

PRIMARY & SECONDARY OUTCOME EVENTS *(For serious adverse events see below)*
Has the patient had an outcome event or died ? Yes (1) No (2) If Yes, date _____/_____/_____
Was the event fatal? Yes (1) No (2)
Nature of the outcome event or death *(please circle one or more):*
Primary:

(1) Ischaemic stroke of the brain
Please circle likely cause:
(a) large artery disease
(b) small vessel disease
(c) embolism from the heart
(d) of unknown or uncertain cause
(2) Ischaemic stroke of the eye
Please circle likely cause:
(a) large artery disease
(b) small vessel disease
(c) embolism from the heart
(d) of unknown or uncertain cause
(3) Haemorrhagic stroke - intracerebral haemorrhage
(4) Haemorrhagic stroke - subarachnoid haemorrhage
(5) Stroke of unknown pathological type
(6) Procedure-related stroke
(7) Myocardial infarction
(8) Other vascular death (eg pulmonary embolus, ruptured aortic aneurysm, heart failure, sudden presumed cardiac death) *Please specify* _____

Secondary:

(9) Non vascular death (*please specify cause of death)* _____
(10) TIA of brain or eye
(11) Revascularization procedures (eg percutaneous transluminal coronary angioplasty [PTCA with or without stent placement], coronary artery bypass surgery, carotid endarterectomy, percutaneous transluminal carotid angioplasty with or without stent replacement, peripheral arterial bypass surgery or any therapeutic intervention for critical leg ischaemia [including toe & leg amputation for PAD]).
Please specify_____
(12) Unstable angina
(13) Pulmonary embolus (non-fatal)
(14) Deep vein thrombosis
(15) Osteoporotic fracture *(Please Circle)* (a) probable or (b) definite
Please Circle Site of Fracture:
(i) neck of femur (ii) distal radius
(iii) thoracic spine (iv) other: please specify _____
(16) Dementia
(17) Depression

SERIOUS ADVERSE EVENTS
(Please notify the trial office immediately on +61 8 9224 3336 of all suspected serious adverse events)
Has the patient experienced a serious adverse event? Yes (1) No (2) If Yes, date _____/_____/_____
Nature of the event: (*please specify)* _____
_____
ICD-10 Category (1-21)_____
Relationship of the serious adverse event to study medication *(please circle):*

(1) Not related
(2) Possibly related
(3) Probably related
(4) Definitely related

Other comments:_____
_____

Please FAX this form now to: VITATOPS Trial coordinator Fax: XXX XXX

VITATOPS Major Events Form *(page 2)*

Please use a black pen

Patient initials: _____ Signature of randomising Dr/RN:_____ VITATOPS number: _____
Date of Randomising event: _____
Please provide the details of any adverse event, primary & secondary outcome events or death below

Date of Onset: _____

Description of Onset:_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Duration: _____
_____

Did patient stop the trial medication at all during this event? Yes (1) No (2)
If yes, specify date patient ceased and recommenced study tablets.

_____

Details:_____
_____
_____
_____
_____
_____
_____

Any precipitating factors:_____
_____
_____
_____
_____

Pathology *(Please FAX a copy of any relevant investigations):*

_____
_____

If the event was a stroke was it a disabling stroke (Rankin ≥ 3) or not (Rankin < 3) on the first day of the stroke?

_____
_____

Please FAX this form now with a copy of all relevant investigations to:
VITATOPS Trial coordinator Fax: XXX XXX

VITATOPS Study - MMSE

Centre and Patient Details:
Patient initials _____ Randomising Dr/RN _____VITATOPS number _____ Today's date ____/_____/_____

Attempted
Orientation:
What is the Year ? Season ? Date ? Day ? Month ? *(score 1 point for each correct answer)* ____/5 ___
What is the name of the Country ? State ? City ? Hospital ? Ward ? *(score 1 point for each correct answer)* ____/5 ___

Registration:
Name three objects: (eg ball, flag, tree) taking one second to say each. Then ask the patient to repeat all three object you have named. *(Score 1 point for each item repeated.)* ____/3 ___
If the patient is not successful, repeat them until he/she learns all three.
(This is preparation for the recall item below).

Attention and calculation:
Ask the patient to subtract 7 from 100, and then 7 from the result – repeat this five times.
(score 1 point each time a correct subtraction is performed) ____/5 ___
Recall:
Ask for the three objects repeated in the registration test. *(score 1 point for each correct answer)* ____/3 ___
Language:
Show a pencil and a watch and ask the subject to name them. *(score 1 point for each correct answer)* ____/2 ____
Repeat the following: "no ifs, ands or buts". *(score 1 point for if answered correctly)* ____/1 ___
A three-stage command, "Take this piece of paper in your right hand; fold it in half and put it on the floor."
(score 1 point for each command performed correctly) ____/3 ____
Point below to "CLOSE YOUR EYES" and ask the patient to obey what is written.
(Score 1 point if performed correctly) ____/1 ____
Ask the patient to write a sentence below.
(Score 1 point if the sentence is sensible and has a verb and a subject) ____/1 ____
Ask the patient to copy the diagram below
(Score 1 point if performed correctly) ____/1 ____

TOTAL SCORE **____/30**

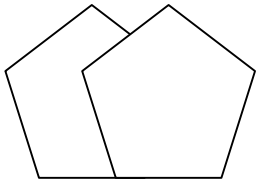
If sensory deficit please complete the following;
MAXIMUM OBTAINABLE SCORE (total points of all items person attempted) ____
MODIFIED SCORE (Total Score x 30 / Maximum Obtainable Score) ____
If sensory deficit, please specify, eg vision _____

If you have any queries please contact the Trial Office on +61 8 9224 7004

CLOSE YOUR EYES

SENTENCE

_____



VITATOPS Study - HAD Scale

Centre and Patient Details:

Patient initials _____ Randomising Dr/RN _____ VITATOPS number _____ Today's date ____/____/_____

Doctors are aware that emotions play an important part in most illnesses. If your doctor knows about these feelings he will be able to help you more. This questionnaire is designed to help your doctor to know how you feel. Read each item and circle the reply which comes closest to how you have been feeling in the past week. Don't take too long over your replies: your immediate reaction to each item will probably be more accurate than a long thought-out response.

	A	D
I feel tense or 'wound up':		
Most of the time		3
A lot of the time		2
Time to time, Occasionally		1
Not at all		0
I still enjoy the things I used to enjoy:		
Definitely as much		0
Not quite so much		1
Only a little		2
Hardly at all		3
I get a sort of frightened feeling as if something awful is about to happen:		
Very definitely and quite badly		3
Yes, but not too badly		2
A little, but it doesn't worry me		1
Not at all		0
I can laugh and see the funny side of things:		
As much as I always could		0
Not quite so much now		1
Definitely not so much now		2
Not at all		3
Worrying thoughts go through my mind:		
A great deal of the time		3
A lot of the time		2
From time to time but not too often		1
Only occasionally		0
I feel cheerful:		
Not at all		3
Not often		2
Sometimes		1
Most of the time		0
I can sit at ease and feel relaxed:		
Definitely		0
Usually		1
Not often		2
Not at all		3

	A	D
I feel as if I am slowed down:		
Nearly all the time		3
Very often		2
Sometimes		1
Not at all		0
I get a sort of frightened feeling like 'butterflies' in the stomach:		
Not at all		0
Occasionally		1
Quite often		2
Very often		3
I have lost interest in my appearance:		
Definitely		3
I don't take so much care as I should		2
I may not take quite as much care		1
I take just as much care as ever		0
I feel restless as if I have to be on the move:		
Very much indeed		3
Quite a lot		2
Not very much		1
Not at all		0
I look forward with enjoyment to things:		
As much as I ever did		0
Rather less than I used to		1
Definitely less than I used to		2
Hardly at all		3
I get sudden feelings of panic:		
Very often indeed		3
Quite often		2
Not very often		1
Not at all		0
I can enjoy a good book or radio or TV program:		
Often		0
Sometimes		1
Not often		2
Very seldom		3

For hospital use only

A _____ D _____

Example letter to the general practitioner

Information for the general practitioner

Dear Colleague,

RE: VITATOPS STUDY VITAmins TO Prevent Stroke

Your patient,...….....Date of Birth.....…….....
has agreed to participate in the VITATOPS study, an international multi-centre trial of secondary prevention in stroke. The aim of this study is to determine whether the addition of multi-vitamins (folate 2 mg, B_6 25 mg, B_{12} 500 μg daily) to best medical / surgical management (including risk factor modification) will reduce the combined incidence of recurrent vascular events (stroke and myocardial infarction) and vascular death in patients with recent stroke or TIA.

Elevated plasma levels of the amino acid homocysteine have recently been associated with the risk of developing vascular disease, including stroke, coronary artery disease and peripheral vascular disease. Raised homocysteine levels are most commonly caused by sub-clinical vitamin deficiency and are highly prevalent in patients with vascular disease, although it also appears to be common in the general community. Plasma levels of homocysteine can effectively be lowered using vitamin supplements, and this raises the possibility that vitamins may become a new, simple, inexpensive and non-toxic therapy for the prevention of vascular disease. However, before this approach can be routinely employed in clinical practice, it needs to be proven beyond doubt that reducing the level of homocysteine in the plasma actually reduces the incidence of vascular events.

The VITATOPS study is a double-blind, randomised clinical trial in which patients with a previous stroke will be treated either with a multivitamin combination or placebo for a period of up to 7 years, with the aim being to prevent recurrent fatal and non-fatal vascular events. Neither we, nor the patient will know which treatment they are receiving, although a special Data and Safety Committee will perform interim analyses on a regular basis to ensure patients safety.

If your patient has an unexpected vascular complication (stroke or myocardial infarction) or dies from whatever cause, we would appreciate it if you would let us know immediately. Likewise, in the event that your patient develops any adverse events which you believe may be related to multi-vitamin therapy (although we do not anticipate any adverse events), we would like to be informed as soon as possible. If your patient requires vitamin therapy for any other reason (eg. megaloblastic anaemia), we also need to be informed.

If you have any questions, we are at all times prepared to discuss these with you. Your questions can be directed either to:

VITATOPS
VITAmins TO Prevent Stroke

Study Coordinator
VITATOPS Trial Office
Royal Perth Hospital
PO Box X2213,
Perth, Western Australia, 6847
Phone +61 (8) 9224 7004/9224 3336
Fax +61 (8) 9224 8424

Dr G Hankey, Study Chairman
Stroke Unit
Royal Perth Hospital
PO Box X2213,
Perth, Western Australia, 6847
Phone +61 (8) 9224 2598
Fax +61 (8) 9224 3323

Example Patient Information Sheet

Invitation

We would like to invite you to join a large study of long-term vitamin therapy to prevent stroke and other forms of blood vessel disease. This study will involve more than 8000 men and women who – like you - have experienced a recent stroke or transient ischaemic attack (TIA).

This invitation is made with the agreement of your doctors. Of course, the decision to join the study is up to you. If you do decide to take part, you are free to withdraw from the study at any time without prejudice to future medical treatment.

Vitamins and Stroke

Each year, 14,000 Australians die as a result of stroke. Those who survive remain at an increased risk of having a second stroke or suffering a heart attack or blocked arteries in the legs. Therefore, one of the most important issues faced by doctors and those who have had a stroke is to prevent further strokes from happening. Unfortunately, the cause of stroke is unclear, so it is difficult to know how to prevent another stroke.

It was recently discovered that high levels of a substance called homocysteine (pronounced `home-o-sis-teen`) in the blood can damage the lining of blood vessels, causing `hardening of the arteries` (atherosclerosis). This eventually causes them to become completely blocked. When such a blockage occurs in the brain, there is poor supply of oxygen to the brain causing a stroke. However, homocysteine levels can be effectively lowered using vitamins, which means we may be able to prevent stroke by taking vitamins. As yet, no one has proven that vitamin treatment prevents stroke and other forms of blood vessel disease. The VITATOPS study has been designed to answer this question.

VITATOPS

VITATOPS is an international study designed to determine whether or not vitamins prevent stroke and other forms of blood vessel disease. People such as yourself who have had a stroke will be treated with either a daily multi-vitamin tablet or placebo (a ‘dummy’ tablet), for 1 to 7 years. During this time, you will be closely monitored by your neurologist, physician or nurse at the VITATOPS clinic. Neither you nor your doctors will know which treatment you are receiving. At the end of the study patients taking vitamins will be compared with those taking the placebo to determine whether vitamin treatment is beneficial or not.

If you decide to join the study you will be asked to take one of the prescribed tablets daily and to return to your neurologist, physician or nurse every six months to assess your progress. We ask you not to take any extra vitamin tablets during this study unless they are prescribed by one of your doctors (in which case we would also like to know), as this may interfere with the results of the VITATOPS Study.

Adverse-effects

All medications, including vitamins, have the potential to cause side effects. The dose of B_6 used in this study (25 mg/day) is not expected to cause any adverse effects. Much higher doses (in excess of 50 mg/day) taken over a long period of time may lead to reduced feeling in the fingers and toes (sensory neuropathy), but the effects have not been reported in lower doses as used here. While the effects of the study drug will be monitored carefully during the trial, if you do experience any side effects it is important to contact your neurologist or physician immediately.

Long-term Participation

If you decide to join the study, you will be committed to take the study treatment for at least one year and up to seven years, with regular visits to the VITATOPS clinic. If you are not willing or able to do this then we would advise you not to join the study. If you do join the study you would, of course, be free to withdraw at any time. However, we would still like to see you regularly to check on your health.

Questions

If you have any questions please ask the VITATOPS clinic staff or your doctor. Alternatively you may direct your questions or any concerns you may have to Clinical Professor J.A. Millar, Chairperson, Ethics Committee, Royal Perth Hospital, Wellington Street, Perth.

VITATOPS Trial Office
Stroke Unit
Royal Perth Hospital
PO Box X2213, Perth
Western Australia, 6847

Phone : +61 (8) 9224 7004 or +61 (8) 9224 3336

HOSPITAL LETTERHEAD

Example consent form

VITATOPS STUDY VITAmins TO Prevent Stroke

Consent Form

I,_____(name),

of_____(address),

have been invited to participate in the **VITA**mins **TO P**revent **S**troke (VITATOPS) Study. In relation to this study, I have been informed of the following points:

1. Approval has been given by the Ethics Committee at this Hospital.

2. This study aims to show whether vitamin treatment will reduce or prevent stroke, heart attack and blockage of blood vessels in the legs in patients who have had a recent stroke.

3. The nature, purpose and effects of this study as far as it affects me, have been fully explained to my satisfaction by the research worker, and my consent is given voluntarily.

4. The results of this study may or may not be of direct benefit to my medical management.

5. I understand that there are some possible side-effects or risks related to this study. These are detailed in the Patient Information Sheet, which I have read.

6. I consent to the Study Investigators having access to my health records (including records held by my general practitioner) to ascertain any present and future health problems.

7. I understand that while information gained from this study may be published, I will not be identified and my personal results will remain confidential.

8. I understand that I can refuse to take part in this study or I can withdraw from this study at any stage and this will not affect my medical care, now or in the future.

9. Should I develop a problem which I suspect might have resulted from my involvement in this study, I am aware that I may contact <insert name and contact for study investigator at this site> or Dr G Hankey +61 8 9224 2598.

10. I understand that if so desired, correspondence regarding any concerns about this study can be directed to Chairperson, Ethics Committee <insert contact name and address>.

SIGNATURE OF PATIENT _____ DATE_____
(or patient's legal representative)

I, _____have described to_____ the research project and the nature and effects of the procedure(s) involved. In my opinion he/she understands the explanation and has freely given his/her consent.

SIGNATURE: _____ DATE: _____