Homocysteine lowering interventions for preventing cardiovascular events

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Abstract

Background—Cardiovascular disease such as coronary artery disease, stroke and congestive heart failure, is a leading cause of death worldwide. A postulated risk factor is elevated circulating total homocysteine (tHcy) levels which is influenced mainly by blood levels of cyanocobalamin (vitamin B12), folic acid (vitamin B9) and pyridoxine (vitamin B6). There is uncertainty regarding the strength of association between tHcy and the risk of cardiovascular disease.

Objectives—To assess the clinical effectiveness of homocysteine-lowering interventions (HLI) in people with or without pre-existing cardiovascular disease.

Search methods—We searched The Cochrane Central Register of Controlled Trials (CENTRAL) on *The Cochrane Library* (issue 3 2008), MEDLINE (1950 to August 2008), EMBASE (1988 to August 2008), and LILACS (1982 to September 2, 2008). We also searched in Allied and Complementary Medicine (AMED; 1985 to August 2008), ISI Web of Science (1993 to August 2008), and the Cochrane Stroke Group Specialised Register (April 2007). We hand searched pertinent journals and the reference lists of included papers. We also contacted researchers in the field. There was no language restriction in the search.

Selection criteria—We included randomised clinical trials (RCTs) assessing the effects of HLI for preventing cardiovascular events with a follow-up period of 1 year or longer. We considered myocardial infarction and stroke as the primary outcomes. We excluded studies in patients with end-stage renal disease.

Data collection and analysis—We independently performed study selection, risk of bias assessment and data extraction. We estimated relative risks (RR) for dichotomous outcomes. We measured statistical heterogeneity using I^2. We used a random-effects model to synthesise the findings.

Main results—We included eight RCTs involving 24,210 participants with a low risk of bias in general terms. HLI did not reduce the risk of non-fatal or fatal myocardial infarction, stroke, or death by any cause (pooled RR 1.03, 95% CI 0.94 to 1.13, $I^2 = 0\%$; pooled RR 0.89, 95% CI 0.73 to 1.08, $I^2 = 15\%$); and pooled RR 1.00 (95% CI 0.92 to 1.09, I^2: 0%), respectively.

Authors' conclusions—Results from available published trials suggest that there is no evidence to support the use of HLI to prevent cardiovascular events.

Medical Subject Headings (MeSH)

Angina Pectoris [prevention & control]; Cardiovascular Diseases [*prevention & control]; Hyperhomocysteinemia [*therapy]; Myocardial Infarction [prevention & control]; Randomized Controlled Trials as Topic; Stroke [prevention & control]; Vitamin B Complex [*therapeutic use]

MeSH check words

Humans

BACKGROUND

Description of the condition

The burden of cardiovascular disease—Cardiovascular disease (CVD) is the number one cause of death worldwide (Jamison 2006; WHO 2002). It covers a wide array of disorders, including diseases of the cardiac muscle and of the vascular system supplying the heart, brain and other vital organs (Gaziano 2006). The most common causes of CVD morbidity and mortality are ischaemic heart disease, stroke, and congestive heart failure (Gaziano 2006).

The burden of CVD is significant and ischaemic heart disease is the single largest cause of death in developed countries. Moreover, it is one of the main contributors to death in developing countries (Table 1; Gaziano 2006). This knowledge is useful when developing strategies for reducing morbidity, mortality and costs (Kahn 2008; Math 2007).

The major risk factors for CVD include tobacco use, high blood pressure, high blood glucose, lipid abnormalities, obesity, and physical inactivity (Epstein 1996; Gaziano 2006; Narayan 2006; Rodgers 2006; WHO 2002; Willet 2006). However, there are other risk factors for CVD called 'emergent or new risk factors' (NACB 2009).

Homocysteine as a risk factor for cardiovascular disease—One of the emergent risk factors is elevated circulating total homocysteine (tHcy) levels. Homocysteine is an amino acid (Hackam 2003; NACB 2009; Nygård 1997; Prasad 1999; Selhub 2006; Ueland 1992) and several observational studies have shown that tHcy is a risk factor for CVD (Casas 2005; Danesh 1998; Davey Smith 2003; Eikelboom 1999; Ford 2002; Guthikonda 2006; Jacobsen 2005; HSC 2002; Nygård 1997; Refsum 1998; Splaver 2004; Stampfer 1992; Wald 2002; Wang 2005). The public significance of raised circulating tHcy has been considered recently (Shelhub 2008).

In 1962, it was hypothesised that increased levels of tHcy might cause vascular disease: the homocysteine theory of arteriosclerosis (McCully 2005). The pathways through which tHcy may cause damage to endothelial cells and lead to atherosclerosis have been widely described (Ferretti 2006; Jacobsen 2006; Jakubowski 2000; Jakubowski 2004; Jakubowski 2008; Obeid 2009; Riksen 2005; Zhou 2009).

Circulating tHcy is composed by protein (albumin)-homocysteine mixed disulfide, sulfhydryl form and low molecular weight disulfides (Mudd 2000). The normal levels of tHcy are close to 10 μmol/L (Mudd 2000). Hyperhomocysteinemia (HHcy) is defined as the presence of an abnormally elevated concentration of plasma or serum tHcy (Mudd 2000). However, there is some controversy to about defining the degree of HHcy. Fasting tHcy concentrations between 12 and 30 μmol/L are commutable termed mild or moderate, while intermediate HHcy includes levels between 31 to 100 μmol/L, and severe HHcy reflects values > 100 μmol/L (Maron 2006). In the general population, the prevalence of HHcy is between 5% and 10% (Refsum 1998). However, rates may be as high as 30-40% in the elderly population (Selhub 1993). According to the results of population-based studies, up to 10% of events due to CAD may be attributable to elevated circulating tHcy levels (Boushey 1995).

Description of the intervention

B-complex vitamins: cyanocobalamin (B12), folic acid (B9) and pyridoxine (B6) as a supplement.

How the intervention might work

The B-complex vitamins are required for transformation, excretion or both steps in the tHcy metabolism pathway (Castro 2006; Fowler 2005; Per a-Kaján 2007; Ramakrishnan 2006). Supplementation with B-complex vitamins reduces the tHcy levels (Clarke 2007; HLTC 2005). There is some ambiguity regarding the function of pyridoxine (vitamin B6): vitamin B6 supplementation has been shown to lower tHcy levels after methionine load, which is an experimental situation and it is believed that it is a weak determinant of circulating tHcy levels. However, at least two studies showed the contrary (Gori 2007; Sofi 2008).

Why it is important to do this review

Elevated circulating tHcy is a powerful prognostic marker of mortality and CVD events in patients with pre-existing CVD risk factors, but evidence is not sufficient to conclude that moderately raised tHcy causes CVD (Refsum 2006). Recently, a meta-analysis concluded

that "each increase of 5 μmol/L in tHcy levels increases the risk of CHD events by approximately 20%, independently of traditional CHD risk factors" (Humphrey 2008).

Many studies have shown that supplementation with B-complex vitamins reduces the circulating tHcy (Folsom 1998; HLTC 2005; Jacques 1999; Robinson 1998; Verhaar 2002). However, there is uncertainty regarding the strength of association between tHcy and the risk of CVD. There is some evidence from controlled trials that the postulated association may be weaker than previously believed (B-VTTC 2006). According to National Institutes of Health the annuals sales of supplements in the United States are at about $23 billion, "a substantial share of which is spent on vitamins and minerals" (NIH 2006). This economic burden could be reduced if the true role of tHcy in CVD is determined. Unfortunately, to our knowledge, we lack the data of the savings which might explain how often these supplements are taken for the specific purpose of lowering tHcy levels.

The first systematic review on this issue included twelve randomised controlled trials (RCT) (16,958 participants) that compared folic acid supplementation with either placebo or usual care for a minimum duration of six months found no benefit of the intervention (Bazzano 2006). Limitations of this systematic review are: it only evaluated a folic acid supplementation; there is a risk of publication bias (only included search in MEDLINE); it evaluated studies with a follow-up period of less than 12 months. A second systematic review assessed the impact of folic acid (vitamin B9) for preventing stroke (Wang 2007). They concluded that the intervention is beneficial in reducing the risk of stroke (RR 0.82, 95% CI 0.68 to 1.00). However, this systematic review did not describe statistical heterogeneity (I^2), it included papers with short follow up (less than six months), the forest plot is absent, sensitivity analysis data were not shown, and the intervention group also included regimens incorporating cyanocobalamin (B12) and pyridoxine (B6) vitamins in combination with folic acid (Wang 2007). Spence 2007 suggested also that folic acid could reduce stroke incidence. His argument was based on HOPE-2 2006 and a sub-study from VISP 2004.

Five RCT comparing B6, B9 and B12 vitamins to placebo demonstrated that homocysteine-lowering interventions (HLI) are not effective for preventing CVD (NORVIT 2006, HOPE-2 2006; VISP 2004; WAFACS 2008; WENBIT 2008). Thus, controversy remains on whether tHcy is or not a marker or causal agent of the CVD (Guilland 2003; Moat 2008; McNulty 2008; Potter 2008b; Ueland 2000).

This review aims to address the question: what is the clinical effectiveness and safety of HLI compared to placebo or low-dose vitamins B6, B9 and B12 for preventing cardiovascular events?

OBJECTIVES

To determine whether HLI in those with and without pre-existing cardiovascular disease:

(a) are effective for preventing cardiovascular events and death (all cause mortality);

(b) are safe for preventing cardiovascular events;

(c) differ in efficacy or safety.

METHODS

Criteria for considering studies for this review

Types of studies—Randomised controlled trials with a follow-up period of 1 year or longer.

Types of participants—Adults (> 18 years) at risk of or with established cardiovascular disease. Studies with patients with end-stage renal disease were excluded.

Types of interventions—The interventions considered were vitamins B6, B9 or B12 given alone or in combination, at any dosage and via any administration route. Comparisons were made with placebo, or with differing regimes of vitamins B6, B9 or B12. When the included population was at risk, combinations of HLI with standard treatment (such as antihypertensives and statins) were considered as long as the same standard treatment was given to the control.

Types of outcome measures

<u>**Primary outcomes:**</u>

(1) Non-fatal or fatal myocardial infarction.

(2) Non-fatal or fatal stroke (ischemic or hemorrhagic).

<u>**Secondary outcomes:**</u>

(1) First unstable angina pectoris episode requiring hospitalisation.

(2) Hospitalisation for heart failure.

(3) Mortality by any cause.

(4) Serious or non-serious adverse events.

We defined serious adverse events according to the International Conference on Harmonisation (ICH) Guidelines (ICH-GCP 1997) as any event that leads to death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation and/or results in persistent or significant disability. All other adverse events will be considered non-serious.

Search methods for identification of studies

We searched the Cochrane Central Register of Controlled Trials (CENTRAL) on *The Cochrane Library* (issue 3 2008), MEDLINE (1950 to August 2008), EMBASE (1988 to the August 2008), LILACS (1982 to September 2, 2008), Allied and Complementary Medicine (AMED; 1985 to August 2008) and ISI Web of Science (1993 to August 2008). The search strategies are in Appendix 1. We searched the Cochrane Heart Group Controlled Trials Register and asked the Cochrane Stroke group and the Cochrane Complementary Medicine

group to check their Specialised Registers. We also checked the reference lists of all trials identified by the above methods. No language restrictions were applied.

The following web sites also were searched: http://www.clinicaltrials.gov (September 2, 2008).

http://controlled-trials.com/isrctn/search.asp (September 2, 2008).

We contacted authors and researchers to obtain further details for unpublished studies.

Data collection and analysis

Two authors, Arturo Martí-Carvajal (AMC) and Ivan Solà (IS) assessed each reference identified by the searches to see if they meet the inclusion criteria. AMC, IS and GS independently assessed the risk of bias of the trials according to the Cochrane Handbook (Higgins 2008). We examined the adequacy of the methods used to generate the allocation sequence; the concealment of allocation; and the level of blinding (clinician, participants). We also evaluated the risk associated with dropouts, as estimated by the percentage of participants lost.

We used the following definitions:

Generation of the allocation sequence—

- Adequate, if the allocation sequence was generated by a computer or random number table. Drawing of lots, tossing of a coin, shuffling of cards, or throwing dice will be considered as adequate if a person who was not otherwise involved in the recruitment of participants performed the procedure.

- Unclear, if the trial was described as randomised, but the method used for the allocation sequence generation was not described.

- Inadequate, if a system involving dates, names, or admittance numbers was used for the allocation of patients. These studies are known as quasi-randomised and will be excluded from the present review when assessing beneficial effects.

Allocation concealment—

- Adequate, if the allocation of patients involved a central independent unit, on-site locked computer, identically appearing numbered drug bottles or containers prepared by an independent pharmacist or investigator, or sealed envelopes.

- Unclear, if the trial was described as randomised, but the method used to conceal the allocation was not described.

- Inadequate, if the allocation sequence was known to the investigators who assigned participants or if the study was quasi-randomised.

Blinding (or masking)—Blinding has been assessed for all main outcomes together and has been characterised as:

- Adequate, if the participants of the trial were blinded to the intervention.

- Unclear, if there is no information on blinding.

- Not performed, if the participants were not blinded to the intervention.

We did not use blinding of outcome assessors as possible source of bias. According to a recent study (Wood 2008), this might be irrelevant when the outcomes are subjective as it is the case in this review.

Follow-up—

- Adequate, if the numbers and reasons for drop-outs and withdrawals in all intervention groups were described and where comparable between groups.

- Unclear, if the report gave the impression that there had been no drop-outs or withdrawals, but it is unclear whether the analysis included missing data in an adequate manner

- Inadequate, if the number or reasons for drop-outs and withdrawals was either unbalanced between groups, differ in reason or was high enough to alter the effect of the intervention. To judge the later, we compared to proportion of dropouts to the event rate.

We grouped the studies into two categories; low risk of bias when the allocation concealment was adequate and participants and the providers of care where blinded to the allocation and high risk of bias otherwise.

AMC, IS and Dimitris Lathyris (DL) extracted data and Georgia Salanti checked for discrepancies.

The first author of the paper was contacted if data were missing. We extracted data on the number of participants by allocated treatment group, irrespectively of compliance and whether or not the participant was later thought to be ineligible or otherwise excluded from treatment or follow up. If we were not able to do so, we recorded for each study whether the results pertained to an intention-to-treat analysis or to available-cases analysis.

We quantified statistical heterogeneity using I^2, which describes the percentage of total variation across studies that is due to heterogeneity rather than sampling error (Higgins 2003). We summarized the findings using a random-effects model, as differences were anticipated in terms of interventions and patients. In case of significant heterogeneity we have planned to identify possible causes of heterogeneity by exploring the effect of participant, intervention and study design characteristics.

Subgroup analysis was performed according to the type of intervention.

We conducted a sensitivity analysis comparing the results using all studies and using only those of low risk of bias. We assessed whether the review was possibly subject to publication bias by using a funnel plot.

RESULTS

Description of studies

See: Characteristics of included studies; Characteristics of excluded studies; Characteristics of ongoing studies.

We identified 4,318 references from our search strategy. From these we excluded 4,299 references after examining the title and abstract because they were not relevant. Full reprints of the remaining 19 references were obtained for more detailed examination. This led to the identification of four further publications from their references lists. We finally included 8 RCT (24,210 participants) in 11 articles published between 2002 to 2008. These are described in Characteristics of included studies. The length of follow-up ranged from 1 to 7.3 years. They varied in size, characteristics of participant populations, duration, drug dosage, and experimental design.

Three studies were classified as ongoing studies (SEARCH 2000, SU.FOL.OM3 2003, VITATOPS 2002). These are described in the Characteristics of ongoing studies.

Included studies—Seven trials were conducted in patients with prior cardiovascular heart disease (CVD) such as coronary artery disease, myocardial infarction, stable angina, unstable angina, stroke, intermittent claudication (CHAOS 2002; FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; WAFACS 2008; WENBIT 2008); and a further study included explicitly patients with history of nondisabling cerebral infarction (VISP 2004).

Seven trials included patients with at least one the following known cardiovascular risk factors: diabetes mellitus, hypertension elevated total cholesterol, current smoking and low HDL cholesterol (FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; VISP 2004; WAFACS 2008; WENBIT 2008). This aspect was unclear for CHAOS 2002. WAFACS 2008 included patients with three or more coronary risk factors. One trial explicitly excluded patients with previously-known HHcy (total plasma homocysteine > 18 μmol/L) (FOLARDA 2004).

HOPE-2 2006 included patients without history of CHD. WAFACS 2008 was conducted only with female patients.

Six trials were conducted with more than 1000 patients (CHAOS 2002; HOPE-2 2006; NORVIT 2006; VISP 2004; WAFACS 2008; WENBIT 2008).

Seven studies were controlled with placebo (CHAOS 2002; HOPE-2 2006; NORVIT 2006; WAFACS 2008; WENBIT 2008) or standard care (FOLARDA 2004; GOES 2003) while one study was a randomised controlled trial (VISP 2004) that compared high versus low dose of HLI.

Three trials only included vitamin B9 as intervention (CHAOS 2002; FOLARDA 2004; GOES 2003). Five trials included B6, B9 and B12 vitamins, in any combination, as intervention (HOPE-2 2006; NORVIT 2006; VISP 2004; WAFACS 2008; WENBIT 2008). Further details can be found in Table 2.

FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; WAFACS 2008, and WENBIT 2008 described lipid-lowering drugs used as concomitant medication.

Two trials (VISP 2004; WAFACS 2008) were conducted in fortified population described as "…nutritional intervention programme with a specifically defined target, and fortified food products are expected to become a main source of the specific added nutrient" (Wirakartakusumah 1998). One further trial was performed with mixed population (HOPE-2 2006), and the rest were carried out in not-fortified population (CHAOS 2002; FOLARDA 2004; GOES 2003; NORVIT 2006; WENBIT 2008).

Seven studies had composite outcomes in their analyses (CHAOS 2002; FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; WAFACS 2008; WENBIT 2008). Four studies included revascularization or other vascular procedure (CHAOS 2002; GOES 2003; WAFACS 2008; WENBIT 2008). Seven included stroke as end point (FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; VISP 2004; WAFACS 2008; WENBIT 2008). All assessed the impact of the intervention in the myocar-dial infarction rates. None of the studies included pectoris angina as component of composite outcomes. (see also in Table 3).

Seven studies reported sample size calculation (FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; VISP 2004; WAFACS 2008; WENBIT 2008). They used 80% or 90% power to detect between 20% and 50% reduction in end points.

Blood concentrations of tHcy at baseline were reported in seven RCTs (CHAOS 2002; GOES 2003; HOPE-2 2006; NORVIT 2006; VISP 2004; WAFACS 2008; WENBIT 2008). Five RCTs reported the tHcy levels at the end follow-up (CHAOS 2002; HOPE-2 2006; NORVIT 2006; VISP 2004; WAFACS 2008). WENBIT 2008 described tHcy levels during intervention: 10.8 (SD, 4.5) μmol/L at baseline versus to 7.6 (SD, 2.2) μmol/L after one year of the intervention (B9 and B12 groups). There were not changes in the B6 and placebo groups. CHAOS 2002 did not report tHcy levels at baseline and end follow-up in the control arm. GOES 2003 reported tHcy levels at baseline and end follow-up only for the intervention arm and not for the control. FOLARDA 2004 did not measure the circulating tHcy levels in any of the groups. Figure 1 shows further details.

Definitions used for defining myocardial infarction, stroke, unstable angina, and death (any) are described in Table 4.

We identified 3 duplicate publications associated to three included studies (for a detailed description see Table 5). GOES 2003 has an associated publication with the results corresponding to 42 months of follow. Spence et al. published one reanalysis for VISP 2004. Finally, WAFACS 2008 trialists published a preliminary report.

Excluded studies—Nine studies did not fulfil inclusion criteria and were excluded (Bazzano 2006; FINEST 2006; Lange 2004; Lonn 2007; PACIFIC 2002; Swiss 2002; Vesin 2007; Wang 2007; Wierzbicki 2007). The reasons for exclusion are described in the Characteristics of excluded studies.

Risk of bias in included studies

The risk of bias in the included trials is summarised in Figure 2 and Figure 3, and detailed in the Characteristics of included studies tables.

Of the included trials, five had an adequate allocation concealment (HOPE-2 2006; NORVIT 2006; VISP 2004; WAFACS 2008; WENBIT 2008). The rest did not provide further information on this domain (CHAOS 2002; FOLARDA 2004; GOES 2003).

Five trials were conducted using blinding for participants and providers of care (HOPE-2 2006; NORVIT 2006; VISP 2004; WAFACS 2008; WENBIT 2008). Regarding CHAOS 2002, we received information from one of the authors that the trial was double blinded.

Effects of interventions

See: Summary of findings for the main comparison

Homocysteine-lowering treatments for preventing cardiovascular events compared to placebo or standard care

Results were based on 24,210 participants, and summarised in the Summary of findings for the main comparison .

Primary outcomes

Myocardial infarction (non-fatal or fatal):

- HLI versus placebo group or conventional care (7 RCTs; 20,561 participants; 1,834 events):

  There was no significant difference in non-fatal or fatal myocardial infarction between intervention and placebo or conventional care groups (RR 1.03, 95% CI 0.94 to 1.13; P =0.50, I^2 = 0%). (CHAOS 2002; FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; WAFACS 2008; WENBIT 2008). (see 'Analysis 1.1').

- HLI (high dose) versus HLI (low dose) (1 study; 3,649 participants; 153 events):

  There was no significant difference in non-fatal or fatal myocardial infarction between intervention and control groups (RR 0.90, 95% CI 0.66 to 1.23, P =0.50). (VISP 2004).(see 'Analysis 1.1').

Stroke (non-fatal or fatal):

- LI versus placebo group (5 RCTs; 18,086 participants; 572 events)

There was no significant difference in non-fatal or fatal stroke between intervention and placebo groups (RR 0.89, 95%CI 0.73 to 1.08, P = 0.45, I^2 = 15%). (FOLARDA 2004; HOPE-2 2006; NORVIT 2006; WAFACS 2008; WENBIT 2008) (see 'Analysis 1.2').

- HLI (high dose) versus HLI (low dose): (1 study; 3,649 participants; 300 events).

There was no significant difference in non-fatal or fatal myocardial infarction between intervention and control groups (RR 1.04, 95% CI 0.84 to 1.29, P =0.73) (VISP 2004), see Analysis 1.2.

Secondary outcomes

First unstable angina pectoris episode requiring hospitalisation: There was no significant difference in first unstable angina pectoris episode requiring hospitalisation between intervention and placebo groups (RR 0.98, 95% CI 0.80 to 1.21, P = 0.87, I^2 = 66%), see Analysis 1.3. This outcome was reported by FOLARDA 2004; HOPE-2 2006; NORVIT 2006; WENBIT 2008 (4 RCTs; 12,644 participants; 1,378 events).

Hospitalisation for heart failure: There was no significant difference in hospitalisation for heart failure between intervention and placebo groups (RR 1.16, 95% CI 0.96 to 1.41, P = 0.13) (5,522 participants; 374 events) (HOPE-2 2006).

Mortality by any cause:

- HLI versus placebo group: (6 RCTs, 18,679 participants; 1986 events).

There was no significant difference in mortality by any cause between intervention and placebo groups (RR 1.00, 95% CI 0.92 to 1.09, P = 0.96, I^2: 0%) (FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; WAFACS 2008; WENBIT 2008), see Analysis 1.4.

- HLI (high dose) versus HLI (low dose): (1 RCT, 3,649 participants; 216 events).

There was no significant difference in mortality for any cause between intervention and control groups (RR 0.86, 95% CI 0.66 to 1.11, P = 0.24) (VISP 2004), see Analysis 1.4.

Funnel plots for myocardial infarction, stroke, and death are shown in Figure 4, Figure 5, Figure 6, respectively. Although the apparent asymmetry in the funnel plots is not necessarily associated with publication bias, there is a mild indication that small negative studies are missing. However, this should not be interpreted as a clear evidence of publication bias, mainly due to small number of included studies.

Serious or non-serious adverse events: Three studies reported data on safety (HOPE-2 2006; NORVIT 2006; WENBIT 2008); they included 12,361 participants, and 996 events. The pooled estimate (RR) for cancer (HOPE-2 2006; NORVIT 2006; WENBIT 2008) was 1.06 (95% CI 0.90 to 1.25, P = 0.49, I^2: 21%), see Analysis 1.5.

Subgroup and sensitivity analysis

The heterogeneity for all main outcomes was low as conveyed by the I^2 values. Moreover, very few studies have been included to allow subgroup analysis. Therefore we did not pursue extensive investigation as previously planned. Due to lack of important heterogeneity fixed and random effects models give the same results for the main outcomes. Sensitivity analysis including only trials of low risk of bias (HOPE-2 2006; NORVIT 2006; WAFACS 2008; WENBIT 2008) gave the following results:

Primary outcomes

Myocardial infarction (non-fatal or fatal): HLI versus placebo group (4 RCTs; 17,803 participants; 1,774 events)

There was no significant difference in non-fatal or fatal myocardial infarction between intervention and placebo groups (RR 1.02, 95% CI 0.93 to 1.13, P = 0.60, I^2 =0%), see Analysis 2.1.

Stroke (non-fatal or fatal): HLI versus placebo group (4 RCTs; 17,803 participants; 571 events)

There was no significant difference in non-fatal or fatal stroke between intervention and placebo groups (RR 0.89, 95% CI 0.72 to 1.09, P = 0.27, I^2 =28%), see Analysis 2.2.

Secondary outcomes

First unstable angina pectoris episode requiring hospitalisation: HLI versus placebo group: (3 RCTs; 14,713 participants; 1364 events).

There was no significant difference in first unstable angina pectoris episode requiring hospitalisation between intervention and placebo groups (RR 1.06, 95% CI 0.79 to 1.42, P = 0.95, I^2: 76%), see Analysis 2.3.

Mortality by any cause: HLI versus placebo group: (4 RCTs, 17,803 participants; 1,947 events).

There was no significant difference in mortality for any cause between intervention and placebo groups (RR 1.00, 95% CI 0.92 to 1.09, P = 0.99, I^2: 0%), see Analysis 2.4.

DISCUSSION

Summary of main results

This review of HLI (B vitamins) for preventing cardiovascular events found eight randomised controlled trials, and their critical appraisal does not support the use of those interventions. We did not find significant differences on incidence of myocardial infarction (fatal or non-fatal), stroke (fatal or non fatal) or mortality by any cause. The data of the included studies (24,210 participants) are very consistent. Participants differed somewhat in cardiovascular risk levels (with established CVD or at high risk of CVD), baseline tHcy levels, access to foods fortified with folic acid or not, different dosages of vitamin and control groups, and treatment periods varying from 2 to 7 years.

Overall completeness and applicability of evidence

From the 1960's, there is a 'homocysteine theory of arteriosclerosis' (McCully 2005). Evidence from several epidemiological observational studies suggested that elevated circulating tHcy levels are related to a higher risk of coronary heart disease, stroke and peripheral vascular disease. However, observational studies could be influenced by known biases. The literature on plausible biological mechanisms for supporting those probable

causal relationship between raised tHcy levels and CVD is abundant and up-to-date (Abahji 2007; Ferretti 2006; Jacobsen 2005; Jakubowski 2000; Jakubowski 2004; Jakubowski 2008; Moens 2007; Riksen 2005; Splaver 2004; Tanriverdi 2007; Wang 2005; Zhou 2009). Further, it has been shown that supplementation with folic acid reduces circulating tHcy levels (Mirarefin 2007; PACIFIC 2002; Sato 2002). As the case might be similar to this previously observed when studying the effect of estrogens (Hulley 1998) and antioxidant vitamins (Vivekananthan 2003) used for preventing cardiovascular disease, randomised evidence is important before establishing the postulated association. All included RCT in this review, each one separately or summarised found not evidence of benefit (Figure 7) (CHAOS 2002; FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; VISP 2004; WAFACS 2008; WENBIT 2008). As emphasised by Dusitanond 2005 referring to a sub-study of an ongoing trial (VITATOPS 2002): "elevated tHcy is a non causal marker of increased vascular risk".

Marcus et al studied the apparent lack of efficacy in "Homocysteine lowering and cardiovascular disease risk: lost in translation" (Marcus 2007). The reasons might be found at the molecular level. Firstly, it might be the autoantibodies against N-homocysteinylated proteins demonstrated in patients with premature coronary artery disease and stroke (Beł towski 2005; Jacobsen 2005; Jakubowski 2005; Per a-Kaján 2007). Undas 2006 showed that tHcy-related autoimmune response is resistant to folic acid administration in patients with coronary heart disease; but in healthy subjects there was a reduction of the circulating tHcy levels with a step down of those antibodies. Secondly, the tHcy-related autoimmune response is caused by a metabolite called homocysteine-thiolactone which modifies proteins such as fibrinogen, low-density lipoprotein, high-density lipoprotein, albumin, haemoglobin, and ferritin and generates that immune response causing autoantibodies against N-homocysteinylated proteins. It enhances thrombosis (Jakubowski 2008; Jakubowski 2009;). Thirdly, high levels of tHcy generate an inflammatory response in endothelial cells, this reaction is mediated by cytokines, molecules that are necessary for inter-cellular communications, and they are involved in the atherothrombotic process (Libby 1995; Poddar 2001; Su 2005).

A research was recently conducted to assess the effect of homocysteine reduction by B-vitamin supplementation on inflammatory markers (Peeters 2007). The study demonstrated a reduction of the tHcy concentration without effect on cytokine levels (Peeters 2007). Therefore, the hypothesis suggesting that high circulating tHcy increases arteriosclerotic or thrombotic risk through vascular inflammation is questionable (Peeters 2007). Similiar findings were found in a sub-study of WENBIT 2008. The authors stated that "homocysteine-lowering therapy with B-vitamins does not affect levels of inflammatory markers associated with atherogenesis" (Bleie 2007). These findings and the results from RCTs had stimulated studies with non-vitamin therapy to reassess the homocysteine theory of atherosclerosis (Urquhart 2007). Lastly, HLI improving effects on endothelial function have been found to be significant in longer trials only (Potter 2008a).

Folic acid fortification is mandatory only in the USA from 1998.The objective was to lower serum homocysteine concentration and vascular disease risk (Koehler 1997). However only minor effect on tHcy-related mortality was observed (Anderson 2004). Also, high folic acid

supplementation could mask the diagnosis of the vitamin B12 deficiency (Rampersaud 2003), a major determinant of elevated tHcy levels and increased carotid plaque area (Robertson 2005). Voluntary fortification policies in healthy adults assessed in Europe showed increased circulating levels of folate related B-complex vitamins and reduced tHcy levels, but possible effects on cardiovascular outcomes were not evaluated (Hoey 2007; Winkels 2008). Only three of the analysed studies in our meta-analysis refer to folate fortification (HOPE-2 2006, VISP 2004, WAFACS 2008). They were all performed in the United States of America. Although, folate fortification reduced the prevalence of low folate levels in the control groups and the levels of homocysteine were either unchanged or reduced, the influence on primary outcomes results (death from cardiovascular causes, myocardial infraction, stroke) was not important. We believe that further evaluation of the effect of folic acid fortification on cardiovascular diseases is necessary.

It is interesting to mention that HOPE-2 2006 study (which showed a significant reduction of stroke) was the only study to use an adequate dose of vitamin B12. The results of this RCT regarding stroke should be interpreted with caution. First, in HOPE-2 2006 combination pills of B6, B9 and B12 vitamins were used, therefore the reduction of stroke events could not be attributed to the adequate dosing of vitamin B12 since it was not delivered alone. Second, authors of HOPE-2 2006 stated that the stroke results should be interpreted with caution as the number of strokes in their study were much lower than the number of coronary events, the confidence intervals around estimated risks were wide, and the results were not adjusted for the multiplicity of outcomes compared.

The VISP subgroup analysis is a post hoc subgroup analysis of a randomised trial and should be interpreted with caution (Spence 2005). It is possible that a particular group of patients may benefit from intake of adequate doses of B6, B9 and B12 but further studies should be performed in order to arrive to conclusive results. Metabolic vitamin B12 deficiency exists in 20% of people over age 65 (Andrès 2004), and is defined either by elevation of methylmalonic acid, or in folate-replete subjects, by elevation of tHcy levels above 14 μmol/L. The VISP subgroup analysis (Spence 2005) , which showed that when patients with vitamin B12 deficiency and those with renal failure were excluded from the analysis showed a 35% reduction of stroke, death and coronary events with high-dose vs low dose therapy, comparing those with adequate vitamin B12 absorption (serum vitamin B12 above the median) with those with vitamin B12 levels below the median. If future studies are to be conducted, they should take into account the key issue of vitamin B12 deficiency which becomes increasingly more common with age, as does stroke.

This review found a non-significantly increased incidence of cancer-associated HLI (HOPE-2 2006; NORVIT 2006; WENBIT 2008). By advice of its safety committee, WENBIT 2008 was stopped because numerous participants were alerted by the increased risk of cancer reported in NORVIT 2006. Biochemical mechanisms for explaining this relationship between vitamin B9 and colorectal cancer had been described (Loscalzo 2006; Riksen 2005, Cole 2007). The role for folate in carcinogenesis required further research (Ulrich 2006).

Ueland and Clarke suggest to "…defer judgment on the clinical and public health relevance of the "homocysteine hypothesis" until the results of the additional ongoing trials of B-vitamins are available" (Clarke 2007; Ueland 2007). Durga 2005 pointed out that " it is prudent for public health policy makers to await trials…before deciding on vitamin B9 fortification for the general population". It is unclear how many participants will be needed in order to accept the evidence that supplementation of vitamin B9 alone or in combination with vitamin B12 and/or vitamin B6 does not prevent cardiovascular events. Similar to the use of streptokinase to prevent death following myocardial infarction (Yusuf 1985), it is possible that the results of the ongoing trials (SEARCH 2000; SU.FOL.OM3 2003; VITATOPS 2002) would reduce the confidence intervals of the pooled estimate for the outcomes of interest; however it is not very likely that they will change the overall conclusion.

Quality of the evidence

Despite the differences in the methodology, participant and doses the results of studies appear to be consistent regarding the effect of the intervention (B-complex vitamins versus placebo group or conventional care) on myocardial infarction, stroke and death. All large studies were at low risk of bias for allocation concealment and blinding and the large total sample size adds confidence to the conclusions of this review.

However, only a few trials clearly described what HHcy is and determined the circulating tHcy levels during the experiment. This review excluded end-stage renal patients as it is addressed in another Cochrane review (Nigwekar 2009). The impact of losses to follow-up was unclear in many trials and the variability of the interventions across trials might limit generalisability.

Potential biases in the review process

Publication bias represents a major threat to the validity of systematic reviews, particularly reviews of small trials. However, this review has a low risk of publication bias, due to the meticulous trial search. Inconclusive studies have been identified and if more had been identified, the conclusions would be even more sceptical regarding the effect of the intervention (Figure 4; Figure 5; Figure 6).

Agreements and disagreements with other studies or reviews

Despite the methodology characteristics differences, this review found the same findings that Bazzano 2006. Also, two studies performed in individuals with advanced chronic kidney disease who had high tHcy levels demonstrated that HLI did not improve survival or reduce the incidence of vascular disease (Jamison 2007; Vianna 2007). This means that in people with variable tHcy levels (low or high), the B-complex vitamin intervention does not supply benefit to reduce the risk of CVD or death. We could conclude that the tHcy hypothesis for atherothrombotic cardiovascular disease has not been validated (Kaul 2006).

The conclusion of this review contradicted by the findings in Wang 2007. This disagreement is due to several methodology differences between both systematic reviews. However, it is remarkable that Wang 2007 only studied stroke as outcome. All of the studies included in

Wang 2007 found no benefit for reducing the risk of stroke. This review also included one RCT that choose "multiple vitamin/mineral supplement" as the intervention and this may be a confounder (Mark 1996). In addition Wang 2007 did not show the individual data from each of the included RCTs. This meant that statistical heterogeneity could not be assessed and information needed to examine methodological quality, to determine its risk of bias, was absent.

AUTHORS' CONCLUSIONS

Implications for practice

This Cochrane review provides evidence that HLI do not prevent cardiovascular events. The results are based on eight studies (24,210 participants) assessing vitamins B6, B9 or B12 (B-complex vitamins), given alone, or in combination, at any dosage compared with placebo or standard care, or with different regimes of vitamins B6, B9 or B12. The included trials did not show a benefit in preventing cardiovascular events in patients at risk of or with prior cardiovascular events. Therefore prescription of these interventions is not justified, unless new evidence from large high quality trials alters this conclusion.

Implications for research

The results from three ongoing trials will help consolidate or challenge the current conclusions regarding the effects of HLI. The association between the apparent lack of effectiveness of the intervention and adequate dosage of vitamin B12 might require further investigation.

Acknowledgments

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON

Homocysteine-lowering treatments for preventing cardiovascular events compared to placebo or standard care

Patient or population: patients with preventing cardiovascular events

Settings: Patients with known risk factors

Intervention: Homocysteine-lowering treatments

Outcomes	Illustrative comparative risks * (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control	Homocysteine-lowering treatments				
Myocardial infarction	**Low risk population**		**RR 1.03** (0.94 to 1.13)	20561 (7 studies)	⊕⊕⊕⊖ **moderate**[1]	
	50 per 1000	**51 per 1000** (47 to 56)				
	High risk population					
	100 per 1000	**103 per 1000** (94 to 113)				
Stroke	**Low risk population**		**RR 0.89** (0.73 to 1.08)	18086 (5 studies)	⊕⊕⊕⊖ **moderate**	
	50 per 1000	**44 per 1000** (37 to 54)				
	High risk population					
	100 per 1000	**89 per 1000** (73 to 108)				
Death by any cause	**Low risk population**		**RR 1** (0.92 to 1.09)	18679 (6 studies)	⊕⊕⊕⊖ **moderate**	
	50 per 1000	**50 per 1000** (46 to 55)				
	High risk population					
	100 per 1000	**100 per 1000** (92 to 109)				
Serious adverse events (Cancer)	**Low risk population**		**RR 1.06** (0.9 to 1.25)	12361 (3 studies)	⊕⊕⊕⊖ **moderate**	
	10 per 1000	**11 per 1000** (9 to 12)				
	High risk population					
	50 per 1000	**53 per 1000** (45 to 62)				

* The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence

High quality: Further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

CHAOS 2002

Methods	Design: randomised, double blind, placebo-controlled trial. Multicentre study. Follow up period: mean of 1.7 years.
Participants	N: 1882 patients randomised (folic acid: 942 versus placebo: 940 patients). Sex: not reported. Age: not reported. Homocysteine levels at baseline (treatment group) (μmol/L): 11.2 ± 6.9 μmol/L Inclusion criteria (one of the following): Positive coronary angiogram; Admission with myocardial infarction or unstable angina. Exclusion criteria: Not reported.
Interventions	Intervention: folic acid 5 mg per day. Control: placebo in addition to usual drugs. Treatment duration: 2 years.
Outcomes	Composite outcome: myocardial infarction, revascularization, death from cardiovascular cause
Notes	Sponsors: Not available. Other: Data not yet fully published. Results on the table corresponds to conference proceedings. Homocysteine levels were only collected in two participating centres

Risk of bias

Item	Authors' judgement	Description
Adequate sequence generation?	Unclear	No information reported about this domain. Described as randomised.
Allocation concealment?	Unclear	No information reported about this domain.
Blinding? All outcomes	Unclear	Described as double blinded. However, the information was obtained from the final report (abstract)
Incomplete outcome data addressed? All outcomes	Unclear	Flow of participants during trial was not reported.

FOLARDA 2004

Methods	Design: randomised, open label, standard care-controlled trial. Multicentre study. Follow-up period: 1 year.
Participants	N: 283 randomised patients (folic acid: 140 versus standard care: 143). Sex (% male): folic acid: 69% versus standard care: 70%. Age (mean): folic acid: 59 years versus standard care: 59 Homocysteine levels at baseline: not reported Inclusion criteria (one of the following): Myocardial infarction. Total cholesterol value at admission or within 24 hours after onset of symptoms: > 6.5 μmol/L (251 mg/dL), Elevation of CK-MB at least 2 times upper the limit of normal function, Markedly increased chest pain lasting more than 30 minutes or classical ECG changes Exclusion criteria: Age under 18 years, Use of lipid lowering agents within the previous 3 months, High triglyceride levels > 4.5 μmol/L, Known familial dyslipidaemia, Low vitamin B12 levels, Hyperhomocysteinaemia (total plasma homocysteine > 18 μmol/L) or a known disturbed methionine loading test (total plasma homocysteine > 47 μmol/L), Severe renal failure (serum creatinine >180 μmol/L), Hepatic disease, Severe heart failure (New York Heart Association class IV), Scheduled percutaneous coronary intervention (PCI) or coronary artery bypass graft (CABG) operation
Interventions	Intervention: Folic acid: 5 mg per day. Treatment was initiated at least 1 day prior for hospital discharge, and no later of 14 days after the myocardial infarction. The treatment continued for 1 year. Patients in this group also received statin therapy (fluvastatin, 40 mg per day). The clinician had at its discretion the prescription of additional prophylactic medication (aspirin, beta-blocking agents, and/or ACE inhibitors) Control: Standard care: Statin therapy (fluvastatin, 40 mg per day). The clinician had at its discretion the prescription of additional prophylactic medication (aspirin, beta-blocking agents, and/or ACE inhibitors) Treatment duration: 1 year.
Outcomes	Cardiovascular death (sudden death, fatal recurrent MI, fatal stroke, and other cardiovascular deaths). Non-cardiovascular death. Recurrent MI. Recurrent ischaemia requiring hospitalisation or revascularization (PCI, CABG)
Notes	Study phase: III Sample calculation a priori: Sample size calculation to detect (80% power and 5% significance level) a 50% reduction in clinical events in that kind of patients, assuming a one-year event rate of 30%. These numbers resulted in an estimation of 120 patients per group. Analyses conducted in ITT basis. Sponsors: AstraZeneca, The Netherlands, Working group on Cardiovascular research (WCN), The Netherlands. One author is an Established Investigator of the Netherlands Heart Foundation Other: Author did not perform homocysteine-level measures during the study

Risk of bias

Item	Authors' judgement	Description
Adequate sequence generation?	Unclear	No complete information reported on this domain. Quote: 'patients were randomised…'
Allocation concealment?	Unclear	No information reported about this domain.
Blinding? All outcomes	No	'… treatment with open label folic acid […] or not'

| Incomplete outcome data addressed? All outcomes | No | 23 patients discontinued treatment and no information is given |

GOES 2003

Methods	Design: randomised, open-label, controlled trial. Single centre study. Follow-up period: 1 year.
Participants	N: 593 randomised patients (folic acid: 300 versus standard care: 293). Sex (% males): folic acid: 76% versus standard care: 80%. Age (mean±SD): folic acid: 64.9± 9.9 versus standard care: 65.5± 9.7 Homocysteine levels at baseline: not reported Inclusion criteria: Myocardial infarction, Coronary artery lesions (>60%) on coronary angiography, Percutaneous coronary intervention (PCI), Coronary artery bypass graft surgery (CABG), Patients had to be stable, with no invasive vascular procedures scheduled, Statin therapy for at least three months, Taking any form of vitamin B-containing medication, regularly or sporadic Exclusion criteria: Age < 18 years, History of low vitamin B12 levels, Therapy for hyperhomocysteinemia, Severe renal failure, or any other treatment for renal disease, Hepatic disease, Severe heart failure (New York Heart Association functional class IV), Serious illness that would exclude follow-up time of at least three years
Interventions	Intervention:folic acid: 0.5 mg per day. Control group: Standard care Intensive follow-up and treatment of risk factors, with counselling provided by a qualified nurse. Statin dosage was increased when necessary. Dietary counselling was provided, and smoking discouraged Treatment duration: not reported.
Outcomes	Primary outcome (composite): Composite: vascular death (sudden death, fatal recurrent MI, fatal stroke, and other cardiovascular deaths), Non cardiovascular death, Recurrent MI; Invasive coronary procedures (PCI, CABG); Cerebrovascular accident or transient ischemic attack; Any other vascular surgery (carotid endarterectomy, abdominal aneurysmectomy, or peripheral vascular surgery including limb amputation for vascular reasons) Secondary: Hospitalization for unstable angina
Notes	Study phase: III Sample size calculation a priori: Sample size calculation (80% power and 5% significance level) to detect a 50% reduction in clinical events in that kind of patients, assuming a two-years event rate of 15%. These numbers resulted in an estimation of 300 patients per group. Analyses conducted in ITT basis Sponsors: Trial with public funding (Stichting Paracard). Other: The trial allowed the entry of patients taking vitamin B supplementation. These patients showed higher levels of serum folate and lower levels of homocysteine

Risk of bias

Item	Authors' judgement	Description
Adequate sequence generation?	Yes	Quote: 'A computer program randomly allocated patients […] to treatment'
Allocation concealment?	Unclear	No information reported about this domain.

Blinding? All outcomes	No	'… treatment with open label folic acid […] or standard care'
Incomplete outcome data addressed? All outcomes	Yes	After randomization, 12 patients per group withdrew the study but were followed and included in the final analysis

HOPE-2 2006

Methods	Design: randomised, double-blind, placebo-controlled trial. Multicentre international study (13 countries; 145 centres). Follow-up period: five years.
Participants	N: 5522 patients randomised (vitamin: 2758 versus group: 2764 patients). Sex (% males): vitamin: 71.1% versus placebo: 72.4%. Age (mean±SD): vitamin: 68.8 ± 7.1 versus placebo: 68.9± 6.8 Homocysteine level at baseline: 12.2 μmol/L (1.6 mg/L) Inclusion criteria: Men and women aged >55 years, Hstory of vascular disease (coronary, cerebrovascular, or peripheral vascular) or diabetes and additional risk factors for atherosclerosis, Irrespective of their homocysteine levels, from countries with mandatory folate fortification of food (Canada and the United States) and countries without mandatory folate fortification (Brazil, western Europe, and Slovakia) Exclusion criteria: Patients taking vitamin supplements containing more than 0.2 mg of folic acid per day
Interventions	Intervention: Multivitamin therapy with 2.5 mg of folic acid, 50 mg of vitamin B6, and 1 mg of vitamin B12 per day Control: Matching placebo daily Treatment duration: 5 years.
Outcomes	Primary outcome (composite): Death from cardiovascular causes, myocardial infarction, stroke Secondary outcomes: Total Ischaemic events (composite of death from cardiovascular causes, myocardial infarction, stroke, hospitalisation for unstable angina, and revascularization), Death from any cause, Hospitalization for unstable angina or congestive heart failure, Revascularization, Incidence and death for cancer. Other outcomes: transient ischaemic attacks, venous thromboembolic events, fractures
Notes	Study phase: III, registered (ClinicalTrials.gov number NCT00106886) Sample calculation a priori: Sample size calculation to detect between a 17 and a 20% (80% and 90% power, respectively) reduction in the risk rate of primary endpoint over 5 years of follow up (assuming an annual event rate of 4% in the placebo group). These numbers resulted in an estimation of 5000 patients. Analyses conducted in ITT basis Sponsors: Public funding (Canadian Institutes of Health Research). The study medication was provide by Jamieson Laboratories. They were not involved in the design, execution, analysis, or reporting of the trial results

Risk of bias

Item	Authors' judgement	Description
Adequate sequence generation?	Unclear	No information reported about this domain.
Allocation concealment?	Yes	Centralised telephone randomisation (accessible 24h a day).
Blinding? All outcomes	Yes	Vitamins manufactured to be indistinguishable in colour, weight or ability to be dissolved in water

| Incomplete outcome data addressed?
All outcomes | Yes | 21 patients in the treatment group and 16 in the placebo not completed the study.
Vital status know for the 99.3% of the sample. |

NORVIT 2006

Methods	Design: Randomised, double-blind, placebo-controlled trial, with factorial design. Multicentre study. Follow-up period: 3.5 years.
Participants	N: 3749 patients randomised (folic acid, vitamins B6 and B12: 937 versus folic acid, vitamin B12: 935 versus vitamin B6: 934 versus placebo: 943) Sex (% male) Folic acid, vitamins B6 and B12: 73% Folic acid, vitamin B12: 74% Vitamin for B6: 73% Placebo: 75% Age (mean±SD, years) Folic acid, vitamins B6 and B12: 63.6±11.9 Folic acid, vitamin B12: 63.2±11.6 Vitamin B6: 62.5±11.7 Placebo: 62.6±11.4 years Inclusion criteria: Men and women aged 30 to 85 years, History of acute myocardial infarction within seven days before randomisation Exclusion criteria: Coexisting disease associated with a life expectancy < 4 years, Prescribed treatment with B vitamins or untreated vitamin B deficiency, Inability to follow the protocol, as judged by the investigator
Interventions	Intervention: Folic acid (group 1): 0.8 mg; vitamin B12: 0.4 mg; vitamin B6: 40 mg per day Folic acid (group 2): 0.8 mg; vitamin B12: 0.4 mg per day Vitamin B6 (group 3): 40 mg per day Control: Placebo. Medication was delivered in single capsules taken once per day. For the first two weeks after study entry patients in groups 1 and 2 received an additional folic acid dose (5 mg) per day, whereas the other two groups received placebo Treatment duration: Not clearly described.
Outcomes	Primary outcome (composite): Recurrent myocardial infarction, stroke, and sudden death attributed to coronary artery disease Secondary outcomes: Myocardial infarction, Unstable angina pectoris requiring hospitalisation, Coronary revascularization with percutaneous coronary intervention or coronary-artery bypass grafting, Stroke, Death from any cause. Incident cases of cancer.
Notes	Study phase: III, registered (ClinicalTrials.gov number NCT00266487) Sample calculation a priori: Sample size calculation to detect a 20% relative reduction in the rate of primary endpoint (assuming a 25% of endpoints in the placebo group). These numbers resulted in an estimation of 3500 patients assuming in 750 primary events The calculation of the sample size was based on data from previous Scandinavian trials, assuming the three-year rate of the primary end point would be 25 percent in the placebo group. The planned enrolment of 3500 patients, with an average follow-up of 3.0 years, was expected to result in 750 primary events and give the study a statistical power of more than 90 percent to detect a 20 percent relative reduction in the rate of the primary end point, given a two-sided alpha value of 0.05 Sponsors: Public and governmental funding. Supported by the Norwegian Research Council, the Council on Health and Rehabilitation, the University of Tromso, the Norwegian Council on Cardiovascular Disease, the Northern Norway Regional Health Authority, the Norwegian Red Cross, the Foundation to Promote Research into Functional Vitamin B12 Deficiency, and an unrestricted private donation

The study medication was provide by Alpharma.
The sponsors had no role in the design, conduct, or reporting of the study

Risk of bias

Item	Authors' judgement	Description
Adequate sequence generation?	Unclear	No information reported about this domain.
Allocation concealment?	Yes	The manufacturer provided study sites centrally blocks of medication assigned in numerical order
Blinding? All outcomes	Yes	Vitamins manufactured to be indistinguishable in colour, weight or ability to be dissolved in water
Incomplete outcome data addressed? All outcomes	Yes	11% of patients stopped the medication. 94% attended the final visit, but data on mortality was available for the entire sample. Incomplete outcome data for 20 patients. Patients that had not completed the planned follow up were followed by phone or consulted for vital status

VISP 2004

Methods	Design: Randomised, double-blind, placebo-controlled trial. Multicentre international study. Follow-up period: 2 years.
Participants	N: 3680 patients randomised (high dose: 1827 versus low dose: 1853 patients) Sex (% male): high dose: 62.3% versus low dose: 62.8%. Age (mean±SD): high dose: 66.4 (10.8) versus low dose: 66.2 (10.8) Inclusion criteria: Nondisabling ischemic stroke(Modified Rankin Stroke Scale 3): Onset 120 days before randomization. Focal neurological deficit of likely atherothrombotic origin, classified as ischemic stroke by questionnaire/algorithm or confirmed as new cerebral infarction consistent with symptoms by cranial computed tomography or brainmagnetic resonance imaging, Total homocysteine level 25th percentile for North American stroke population, Age: 35 years, Accessibility for follow-up, Agreement to take study medication and not take other multivitamins or pills containing folic acid or vitamin B6, Written informed consent. Exclusion criteria: Potential sources of emboli (atrial fibrillation within 30 days of stroke, prosthetic cardiac valve, intracardiac thrombus or neoplasm, or valvular vegetation), Other major neurological illness that would obscure evaluation of recurrent stroke, Life expectancy 2 years, Renal failure requiring dialysis, Untreated anemia or untreated vitamin B12 deficiency, Systolic blood pressure 185mmHg or diastolic blood pressure 105 mm Hg on 2 readings 5 minutes apart at time of eligibility determination, Refractory depression, severe cognitive impairment, or alcoholism or other substance abuse, Use within the last 30 days of medications that affect total homocysteine level (methotrexate, tamoxifen, levodopa, niacin, or phenytoin) or bile acid sequestrants that can decrease folate levels, Childbearing potential, Participation in another trial with active intervention, General anesthesia or hospital stay of 3 days, any type of invasive cardiac instrumentation, or endarterectomy, stent placement, thrombectomy, or any other endovascular treatment of carotid artery within 30 days prior to randomization or scheduled to be performed within 30 days after randomization
Interventions	High dose multivitamin therapy

2.5 mg folic acid; 0.4 mg vitamin B12; 25 mg vitamin B6 per day
Low dose multivitamin therapy
20 micrograms folic acid; 6 micrograms vitamin B12; 200 micrograms vitamin B6 per day
Co-interventions:
Risk factor control education
Aspirin (325 mg/d).
Duration Treatment: Not described

Outcomes	Primary outcome: Recurrent cerebral infarction. Secondary outcomes: Coronary heart disease, including: myocardial infarction requiring hospitalisation; coronary revascularization; and fatal coronary heart disease Death.
Notes	Study phase: III Sample size calculation a priori: Sample size calculation (80% power a .05 significance level for a 2-sided test) to detect a 30% reduction in the rate of primary endpoint over 2 years of follow up (assuming a 8% of events in the first year and a 4% at the second year, with a 20% of losses of follow up). These numbers resulted in an estimation of 1800 patients per group. Trialists planned up to 6 interim analyses Sponsors: Supported by the National Institute of Neurological Disorders and Stroke (grant RO1 NS34447). The study medication was provided by Roche Inc. They had no role in the design and conduct of the study; the collection, analysis, and interpretation of the data; or the preparation, review, or approval of the manuscript Other: Elegible patients were tested for treatment compliance giving a 1-month low dose vitamins. Only persons taking at least 75% of treatment were randomised

Risk of bias

Item	Authors' judgement	Description
Adequate sequence generation?	Unclear	The allocation of participants was programmed by the statistical coordinating center, encrypted, and entered into a data entry program installed on a studycomputer at each site
Allocation concealment?	Yes	Allocation programmed by the statistical coordinating centre. All the information on assignment were encrypted an entered in computers in study sites. After verification of eligibility participants were assigned in 1 of 20 medication codes
Blinding? All outcomes	Yes	The drug distributor centre bottled and distributed the vitamins, that were manufacture to be indistinguishable in colour, weight or ability to be dissolved in water The primary endpoint was reviewed by a local neurologist and two external independent review neurologists
Incomplete outcome data addressed? All outcomes	Unclear	132 patients in the low dose group, and 133 in the high dose were lost to follow up. Of these 18 and 13 patients respectively had no contact after randomisation, and were not included in the analysis. 186 patients in the low dose group, and 179 in the high dose discontinued the assigned treatment Patients that had not completed the planned follow up were invited to an exit visit

WAFACS 2008

Methods	Design: Randomised, double-blind, placebo-controlled trial, with factorial design Multicentre study Follow-up period: 7.3 years.
Participants	N: 5442 randomised patients (vitamin group: 2721 patients; placebo group: 2721 patients) Sex: Women health professionals Age (mean [Standar deviation])] years

Active group: 62.8 (8.8).
Control group: 62.8 (8.8).
Inclusion criteria
1.- Women
2. Age: 40 years or older
3.- Postmenopausal or had no intention of becoming pregnant
4.- History of CVD or had at least 3 cardiac risk factors
Exclusion criteria:
1.- Cancer (excluding nonmelanoma skin cancer) within the past 10 years.
2.- Serious non-cardiovascular disease.
3.- Warfarin or other anticoagulants use.

Interventions	Intervention: Folic acid: 2.5 mg; vitamin B12: 1 mg; vitamin B6: 50 mg per day Control: Matching placebo per day. Co-interventions: vitamin C, vitamin E, ß-carotene. Treatment duration: not clearly reported.
Outcomes	Primary outcome (composite): Incident myocardial infarction, stroke, coronary revascularization procedures (coronary artery bypass grafting or percutaneous coronary intervention) and cardiovascular mortality Secondary outcomes: Myocardial Infarction rate, Stroke rate, Total coronary heart disease events (MI, coronary revascularization, and death from coronary heart disease)
Notes	Study phase: III, registered (ClinicalTrials.gov number NCT00000541) The information in this table was kindly supplied by Dr. Nancy Cook who was the statistician for the WACS and WAFACS studies (June 23, 2008) The WACS study was a 2×2×2 factorial trial of three anti-oxidants, vitamins C, E and beta-carotene. Randomization of the 8171 participants into the 8 treatment groups took place from June 1995 to October 1996, and was conducted using blocks of size 16 within 5-year age groups. The folate/B6/B12 arm was added in April 1998, and the 5442 participants who were willing and eligible were randomised (at one time) using blocks of size 8 within strata defined by age and the other treatment arms. Participants were sent yearly supplies of calendar packs containing the study medications or matching placebo pills that were identical in appearance. All medical records were reviewed by an Endpoints Committee that was blinded to treatment assignment Sample calculation a priori: Sample size with a 91.5% power to detect a 20% reduction in in the primary end point (major vascular events). For the end points of total CHD (defined as nonfatal MI, CHD death, or revascularization), MI, and stroke, the minimum detectable risk reduction with 80% power ranges from 19% to 32%. It was used a two sided significance level of 0.05 Sponsor: Public funding and from several industry sources. Grant HL47959 from the National Heart, Lung, and Blood Institute of the National Institutes of Health. Vitamin E and its placebo were supplied by Cognis Corporation (La-Grange, Illinois) All other agents and their placebos were supplied by BASF Corporation (Mount Olive, New Jersey). Pill packaging was provided by Cognis and BASF. They did not participate in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript Other: The analyses of the end points were done only for those confirmed outcomes. However, there was an additional 43 records deaths for total mortality

Risk of bias

Item	Authors' judgement	Description
Adequate sequence generation?	Unclear	Blocks randomisation with a size of 8 generated by computer, stratified by age
Allocation concealment?	Yes	Central randomisation. Patients were sent yearly supplies of calendar packs containing their medication or matching placebos identical in appearance
Blinding? All outcomes	Yes	All study investigators, personnel, and participants were unaware of the participants' treatment assignments'. Patients were sent packs containing medication or matching placebos identical in appearance

		An independent committee monitored the 'safety and overall quality and scientific integrity' of the trial, that was blinded to treatment assignment. All the information was supplied by Nancy Cook (WACS statistician, June 23th, 2008)
Incomplete outcome data addressed? All outcomes	Unclear	Unknown vital status for 194 patients in the folic acid group and for 207 patients in the placebo group. All the patients were included in the primary analysis, but not described how

WENBIT 2008

Methods	Design: Randomised, double-blind, placebo-controlled trial, with factorial design Multicentre study. Follow-up period: 4 years.
Participants	N: 3096 patients randomised (folic acid, vitamins B6 and B12: 772 versus folic acid, vitamin B12: 772 versus vitamin B6: 772 versus placebo: 780) Sex (% male) Folic acid, vitamins B6 and B12: 81.2% Folic acid, vitamin B12: 80.4% Vitamin B6: 80.2% Placebo: 76.5% Age (mean±SD, years): Folic acid, vitamins B6 and B12: 61.7±10.3 Folic acid, vitamin B12: 61.3±10.0 Vitamin B6: 61.4±9.7 Placebo: 62.0±9.9 Inclusion criteria: Age: 18 years or older, Undergoing coronary angiography for suspected CAD and/or aortic valve stenosis at the 2 university hospitals in western Norway Exclusion criteria: Unavailability for follow-up, Participation in other trials, Hisytory of alcohol abuse, serious mental illness, or cancer
Interventions	Intervention: Folic acid (group 1): 0.8 mg; vitamin B12: 0.4 mg; vitamin B6: 40 mg per day Folic acid (group 2): 0.8 mg; vitamin B12: 0.4 mg per day Vitamin B6 (group 3): 40 mg per day Control: Placebo. Co-interventions: statins, insulin, aspirin, clopidogrel, Beta-Blockers, ACE inhibitors/ARBs, calcium channel blockers, loop diuretics, oral antidiabetics, medication for Chronic Obstructive Pulmonar Disease Duration Treatment: Not described.
Outcomes	Primary outcome (composite): All-cause death, nonfatal acute myocardial infarction, acute hospitalisation for unstable angina pectoris, and nonfatal thromboembolic stroke Secondary outcomes: Acute Myocardial Infarction, Acute hospitalisation for angina pectoris, Stable angina pectoris with angiographically verified progression, Myocardial revascularization procedures, Stroke, Incident cases of cancer.
Notes	Study phase: III, registered (ClinicalTrials.gov number NCT00354081) Sample calculation a priori: Sample of 3088 participants to detect a 20% reduction in the primary end point during 4 years of follow-up with a statistical power of 80% at a 2-sided significance level of .05 Sponsors: the Advanced Research Program and Research Council of Norway, the Norwegian Foundation for Health and Rehabilitation, the Norwegian Heart and Lung Patient Organisation, the Norwegian Ministry of Health and Care Services, the Western Norway Regional Health Authority, the Department of Heart Disease at

Haukeland University Hospital, Locus for Homocysteine and Related Vitamins at the University of Bergen, Locus for Cardiac Research at the University of Bergen, the Foundation to Promote Research Into Functional Vitamin B12 Deficiency, Bergen, Norway, and Alpharma Inc, Copenhagen, Denmark

The study medication was provide by Alpharma which had no access to study data, and did not participate in data analysis or interpretation or in the preparation, review, or approval of the manuscript

Other: The first 90 participants were randomised before undergoing angiography in order to ensure no effects on blood indexes from the invasive procedure. Subsequent participants were randomised after baseline angiography

This trial was stopped by no beneficial effects and suggesting increased risk of cancer by B vitamin treatment

Risk of bias

Item	Authors' judgement	Description
Adequate sequence generation?	Unclear	2×2 factorial design with block randomisation with a size of 20
Allocation concealment?	Yes	Centralised independently by the manufacturer (Alpharma). Study nurses received coded boxes provided to participants in numerical order. The codes were kept by the manufacturer until eligibility data was complete
Blinding? All outcomes	Yes	Vitamins manufacture to be indistinguishable in colour, weight or ability to be dissolved in water. Endpoints adjudicated by an independent committee unaware of patient's assignment
Incomplete outcome data addressed? All outcomes	No	Data on 6 patients (0.2% from the sample) withdrew consent to participate in the trial and were excluded from the analysis. Due to the media impact of the NORVIT interim results 692 patients were asked to stop the medication. Outcome data available for the 86% of patients at the final visit

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Bazzano 2006	Systematic review.
FINEST 2006	RCT with a follow up less than one year.
Lange 2004	RCT with a follow up less than one year.
Lonn 2007	Review.
PACIFIC 2002	RCT with a follow up lesser than one year.
Swiss 2002	RCT with a follow less than one year.
Vesin 2007	Review.
Wang 2007	Systematic review.
Wierzbicki 2007	Review.

RCT: randomised controlled trial

Characteristics of ongoing studies *[ordered by study ID]*

SEARCH 2000

Trial name or title	SEARCH: Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine
Methods	
Participants	Ages: 18 to 80 years. Genders: both Inclusion criteria: Prior myocardial infarction; statin therapy indicated; no clear indication for folic acid. Exclusion criteria: No clear contraindication to study treatments; Screening plasma total cholesterol <3.5 μmol/L in patient already on statin therapy, or <4.5 μmol/L in patient not on statin therapy; Chronic liver disease; Severe renal disease or evidence of renal impairment ; Inflammatory muscle disease; Concurrent treatment with fibrates or high-dose niacin; Concurrent treatment with cyclosporin (or condition likely to result in organ transplantation and the need for cyclosporin), nefazodone, methotrexate, systemic azole antifungal or systemic macrolide antibiotics; child bearing potential; No other predominant medical problem (other than coronary heart disease [CHD]) which might limit compliance with 5 years of study treatment
Interventions	Simvastatin (20mg versus 80mg daily). Folic acid 2mg plus vitamin B12 1mg daily.
Outcomes	Primary: Major vascular events (MVE): non-fatal myocardial infarction, coronary death, stroke or arterial revascularization Secondary: MVEs separately in year 1 and in later years; MVEs in patients subdivided into 3 groups by baseline low-density lipoprotein (LDL); MVEs in presence and absence of the other factorial treatment ; major coronary events; total strokes
Starting date	July 1998
Contact information	Rory Collins, MB BS FRCP, Study Director, University of Oxford
Notes	Region: United Kingdom (Oxford). Study Design: prevention, randomised, double-Blind, dose comparison, factorial assignment, safety/efficacy study This study is no longer recruiting patients. Total enrolment: 12064. Phase III. ClinicalTrials.gov Identifier: NCT00124072. Sponsors and collaborators: University of Oxford; Merck.

SU.FOL.OM3 2003

Trial name or title	Double-blind randomised placebo-controlled secondary prevention trial to test the impact of supplementation with folate, vitamin B6 and B12 and/or omega-3 fatty acids on the prevention of recurrent ischemic events in subjects with atherosclerosis in the coronary or cerebral arteries
Methods	
Participants	Patients with myocardial infarction, unstable angina pectoris or ischemic stroke. Population without mandatory folate fortification of food. Age: 45 and 80 years
Interventions	Folate plus vitamin B6 and B12 and/or omega-3 fatty acids and/or placebo
Outcomes	Recurrent ischemic diseases.
Starting date	2003
Contact information	Galan P (s_galan@vcnam.cnam.fr) Unité de Surveillance et d'Epidemiologie Nutritionnelle, Institut Scientifique et Technique de la Nutrition et de l'Alimentation, Paris
Notes	* Study design: Double-blind randomised placebo-controlled. Region: France. Total enrolment: 3,000 patients. Follow-up: 5 years.

Sources: Faeh D et al. SWISS MED WKLY 2006;136:745-756.
Galan P et al. J Nutr Health Aging. 2003;7(6):428-35.

VITATOPS 2002

Trial name or title	A Study of VITAmins TO Prevent Stroke
Methods	
Participants	Patients with stroke or TIA in the previous 7 months. Inclusion Criteria: Patients presenting within seven months of stroke (Ischaemic or hemorrhagic) or TIA. Agree to take study medication Be geographically accessible for follow-up. Provide written informed consent. Exclusion Criteria: Taking folic acid or B6 on medical advice. Use of vitamin supplements containing folate, B6 or B12 (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.
Interventions	Folic acid: 2mg Vitamin B6: 25mg Vitamin B12: 500 micrograms
Outcomes	Primary: Non-fatal stroke Non-fatal myocardial infarction Death due to vascular causes. Secondary: TIA Revascularization procedures Dementia Depression.
Starting date	November 1998
Contact information	Julia Pizzi +61 8 9224 7004 VITATOPS@health.wa.gov.au Michelle Tang +61 8 9224 3336 VITATOPS@health.wa.gov.au
Notes	Region: Australia: Study Design: prevention, randomised, double-blind, placebo control, parallel assignment, safety/efficacy study Sample size calculation: To reliably identify a 15% reduction in relative risk of the primary outcome event from 8% to 6.8% per year with an alpha of 0.05 and power of 80%, 8,000 patients need to be randomised and followed-up for an average of two years Current progress: As of November, 2004, more than 4,400 patients have been randomised in 73 centres in 19 countries in five continents: Australia, Austria, Belgium, Brazil, Hong Kong, Italy, Malaysia, Moldova, Netherlands, New Zealand, Pakistan, Philippines, Portugal, Republic of Georgia, Serbia & Monte Negro, Singapore, Sri Lanka, United Kingdom, and United States

* Authors asked to the author for getting complete data; however, they did not send the information.

NA: not available.

DATA AND ANALYSES

Comparison 1

Homocysteine-lowering treatment versus other (any comparisons)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Myocardial infarction	8		Risk Ratio (M-H, Random, 95% CI)	Subtotals only

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1 Homocysteine-lowering versus placebo	7	20561	Risk Ratio (M-H, Random, 95% CI)	1.03 [0.94, 1.13]
1.2 Homocysteine-lowering treatment at high dose versus low dose	1	3649	Risk Ratio (M-H, Random, 95% CI)	0.90 [0.66, 1.23]
2 Stroke	6		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
2.1 Homocysteine-lowering treatment versus placebo	5	18086	Risk Ratio (M-H, Random, 95% CI)	0.89 [0.73, 1.08]
2.2 Homocysteine-lowering treatment at high dose versus low dose	1	3649	Risk Ratio (M-H, Random, 95% CI)	1.04 [0.84, 1.29]
3 First unstable angina pectoris episode requiring hospitalization	4	12644	Risk Ratio (M-H, Random, 95% CI)	0.98 [0.80, 1.21]
4 Death by any cause	7		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.1 Homocysteine-lowering treatment versus placebo	6	18679	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.92, 1.09]
4.2 Homocysteine-lowering treatments at high dose versus low dose	1	3649	Risk Ratio (M-H, Random, 95% CI)	0.86 [0.66, 1.11]
5 Serious adverse events (Cancer)	3	12361	Risk Ratio (M-H, Random, 95% CI)	1.06 [0.90, 1.25]

Comparison 2

Homocysteine-lowering treatment versus other (Sensitivity analysis)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Myocardial infarction	4	17803	Risk Ratio (M-H, Random, 95% CI)	1.02 [0.93, 1.13]
1.1 Trials with low risk of bias (mixed populations)	3	12361	Risk Ratio (M-H, Random, 95% CI)	1.04 [0.94, 1.15]
1.2 Trials with low risk of bias (only women included)	1	5442	Risk Ratio (M-H, Random, 95% CI)	0.88 [0.63, 1.22]
2 Stroke	4	17803	Risk Ratio (M-H, Random, 95% CI)	0.89 [0.72, 1.09]
2.1 Trials with low risk of bias (mixed populations)	3	12361	Risk Ratio (M-H, Random, 95% CI)	0.79 [0.65, 0.97]
2.2 Trials with low risk of bias (only women included)	1	5442	Risk Ratio (M-H, Random, 95% CI)	1.14 [0.83, 1.57]
3 First unstable angina pectoris episode requiring hospitalization	3	12361	Risk Ratio (M-H, Random, 95% CI)	0.99 [0.79, 1.24]
4 Death by any cause	4	17803	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.92, 1.09]
4.1 Trials with low risk of bias (mixed populations)	3	12361	Risk Ratio (M-H, Random, 95% CI)	1.01 [0.91, 1.12]
4.2 Trials with low risk of bias (only women included)	1	5442	Risk Ratio (M-H, Random, 95% CI)	0.98 [0.83, 1.15]

Analysis 1.1. Comparison 1 Homocysteine-lowering treatment versus other (any comparisons), Outcome 1 Myocardial infarction

Review: Homocysteine lowering interventions for preventing cardiovascular events

Comparison: 1 Homocysteine-lowering treatment versus other (any comparisons)

Outcome: 1 Myocardial infarction
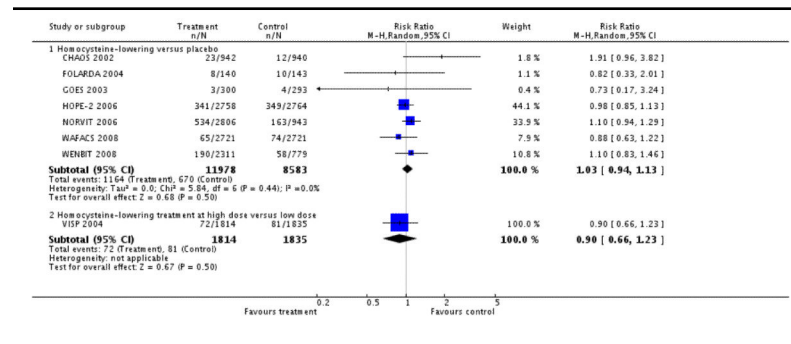
Analysis 1.2. Comparison 1 Homocysteine-lowering treatment versus other (any comparisons), Outcome 2 Stroke

Review: Homocysteine lowering interventions for preventing cardiovascular events

Comparison: 1 Homocysteine-lowering treament versus other (any comparisons)

Outcome: 2 Stroke

Analysis 1.3. Comparison 1 Homocysteine-lowering treatment versus other (any comparisons), Outcome 3 First unstable angina pectoris episode requiring hospitalization

Revies: Homocysteine lowering interventions for preventing cardiovascular events

Comparison: 1 Homocysteine-lowering treatment versus other (any comparisons)

Outcome: 3 First unstable angina pectoris episode requiring hospitalization

Study or subgroup	Treatment n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
FOLARDA 2004	6/140	8/143		3.7 %	0.77 [0.27, 2.15]
HOPE-2 2006	268/2758	219/2764		33.6 %	1.23 [1.03, 1.45]
NORVIT 2006	356/2806	132/943		32.3 %	0.91 [0.75, 1.09]
WENBIT 2008	280/2311	109/779		30.4 %	0.87 [0.70, 1.06]
Total (95% CI)	**8015**	**4629**		**100.0 %**	**0.98 [0.80, 1.21]**

Total events: 910 (Treatment), 468 (Control)
Heterogeneity: Tau² = 0.03; Chi² = 8.72, df = 3 (P = 0.03); I² =66%
Test for overall effect: Z = 0.16 (P = 0.87)

0.1 0.2 0.5 1 2 5 10
Favours treatment   Favours control

Analysis 1.4. Comparison 1 Homocysteine-lowering treatment versus other (any comparisons), Outcome 4 Death by any cause

Review: Homocysteine lowering interventions for preventing cardiovascular events

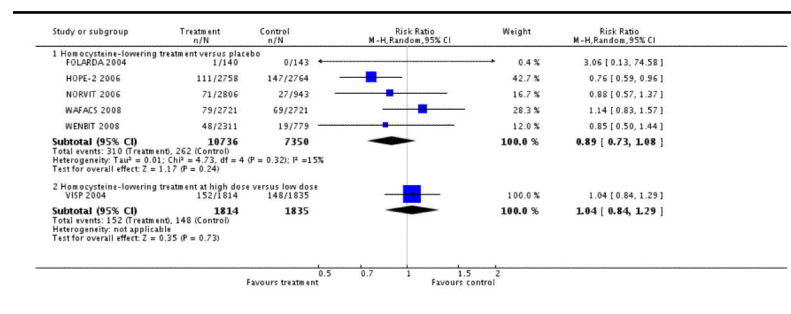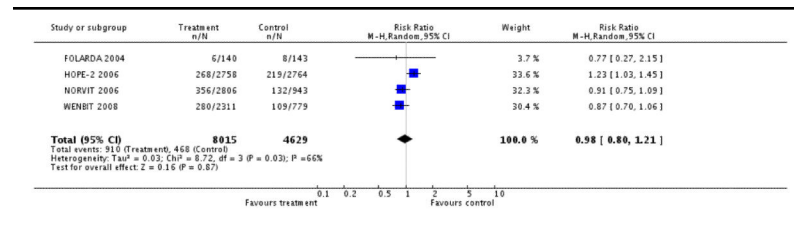Comparison: 1 Homocysteine-lowering treatment versus other (any comparisons)

Outcome: 4 Death by any cause

Study or subgroup	Treatment n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Homocysteine-lowering treatment versus placebo					
FOLARDA 2004	6/140	7/143		0.6 %	0.88 [0.30, 2.54]
GOES 2003	12/300	14/293		1.3 %	0.84 [0.39, 1.78]
HOPE-2 2006	470/2758	475/2764		53.5 %	0.99 [0.88, 1.11]
NORVIT 2006	276/2806	89/943		14.0 %	1.04 [0.83, 1.31]
WAFACS 2008	250/2721	256/2721		26.2 %	0.98 [0.83, 1.15]
WENBIT 2008	101/2311	30/779		4.5 %	1.13 [0.76, 1.69]
Subtotal (95% CI)	**11036**	**7643**		**100.0 %**	**1.00 [0.92, 1.09]**
Total events: 1115 (Treatment), 871 (Control)					
2 Homocysteine-lowering treatments at high dose versus low dose					
VISP 2004	99/1814	117/1835		100.0 %	0.86 [0.66, 1.11]
Subtotal (95% CI)	**1814**	**1835**		**100.0 %**	**0.86 [0.66, 1.11]**

Heterogeneity: Tau² = 0.0; Chi² = 0.88, df = 5 (P = 0.97); I² =0.0%
Test for overall effect: Z = 0.05 (P = 0.96)

Total events: 99 (Treatment), 117 (Control)
Heterogeneity: not applicable
Test for overall effect: Z = 1.17 (P = 0.24)

0.1 0.2 0.5 1 2 5 10
Favours treatment   Favours control

Analysis 1.5. Comparison 1 Homocysteine-lowering treatment versus other (any comparisons), Outcome 5 Serious adverse events (Cancer)

Review: Homocysteine lowering interventions for preventing cardiovascular events

Comparison: 1 Homocysteine-lowering treatment versus other (any comparisons)

Outcome: 5 Serious adverse events (Cancer)

Study or subgroup	Treatment n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
HOPE-2 2006	358/2758	340/2764		66.0 %	1.06 [0.92, 1.21]
NORVIT 2006	104/2806	40/943		18.1 %	0.87 [0.61, 1.25]
WENBIT 2008	123/2311	31/779		15.9 %	1.34 [0.91, 1.97]
Total (95% CI)	**7875**	**4486**		**100.0 %**	**1.06 [0.90, 1.25]**

Total events: 585 (Treatment), 411 (Control)
Heterogeneity: Tau² = 0.01; Chi² = 2.53, df = 2 (P = 0.28); I² =21%
Test for overall effect: Z = 0.68 (P = 0.49)

0.1   0.2   0.5   1   2   5   10
Favours treatment   Favours control

Analysis 2.1. Comparison 2 Homocysteine-lowering treatment versus other (Sensitivity Analysis), Outcome 1 Myocardial infarction

Review: Homocysteine lowering interventions for preventing cardiovascular events

Comparison: 2 Homocysteine-lowering treatment versus other (Sensitivity analysis)

Outcome: 1 Myocardial infarction

Study or subgroup	Treatment n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Trials with low risk of bias (mixed populations)					
HOPE-2 2006	341/2758	349/2764		45.6 %	0.98 [0.85, 1.13]
NORVIT 2006	534/2806	163/943		35.1 %	1.10 [0.94, 1.29]
WENBIT 2008	190/2311	58/779		11.1 %	1.10 [0.83, 1.46]
Subtotal (95% CI)	**7875**	**4486**		**91.8 %**	**1.04 [0.94, 1.15]**
Total events: 1065 (Treatment), 570 (Control)					
Heterogeneity: Tau² = 0.0; Chi² = 1.38, df = 2 (P = 0.50); I² =0.0%					
Test for overall effect: Z = 0.76 (P = 0.44)					
2 Trials with low risk of bias (only women included)					
WAFACS 2008	65/2721	74/2721		8.2 %	0.88 [0.63, 1.22]
Subtotal (95% CI)	**2721**	**2721**		**8.2 %**	**0.88 [0.63, 1.22]**
Total events: 65 (Treatment), 74 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.77 (P = 0.44)					
Total (95% CI)	**10596**	**7207**		**100.0 %**	**1.02 [0.93, 1.13]**
Total events: 1130 (Treatment), 644 (Control)					
Heterogeneity: Tau² = 0.0; Chi² = 2.30, df = 3 (P = 0.51); I² =0.0%					
Test for overall effect: Z = 0.51 (P = 0.61)					

0.1   0.2   0.5   1   2   5   10
Favours treatment   Favours control

Analysis 2.2. Comparison 2 Homocysteine-lowering treatment versus other (Sensitivity Analysis), Outcome 2 Stroke

Review: Homocysteine lowering interventions for preventing cardiovascular events

Comparison: 2 Homocysteine-lowering treatment versus other (Sensitivity analysis)

Outcome: 2 Stroke

Analysis 2.3. Comparison 2 Homocysteine-lowering treatment versus other (Sensitivity Analysis), Outcome 3 First unstable angina pectoris episode requiring hospitalization
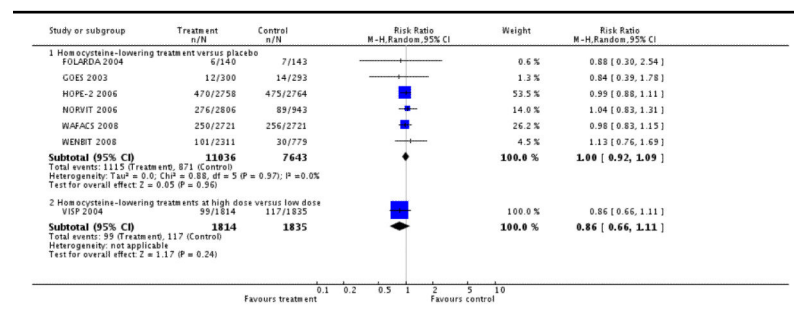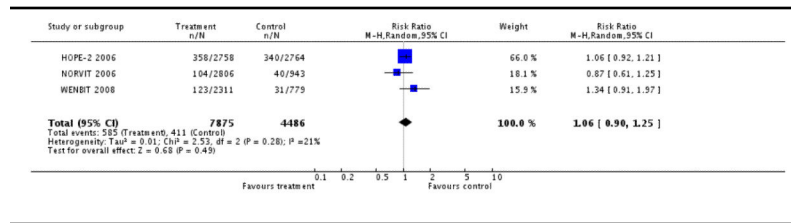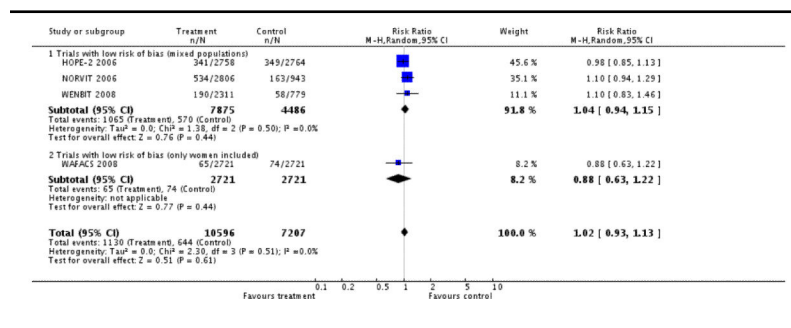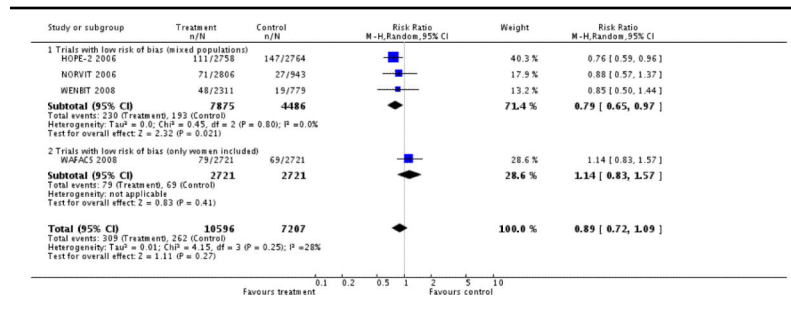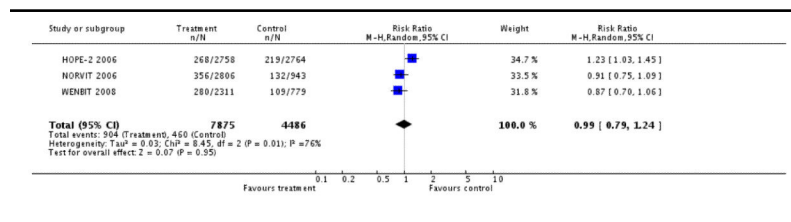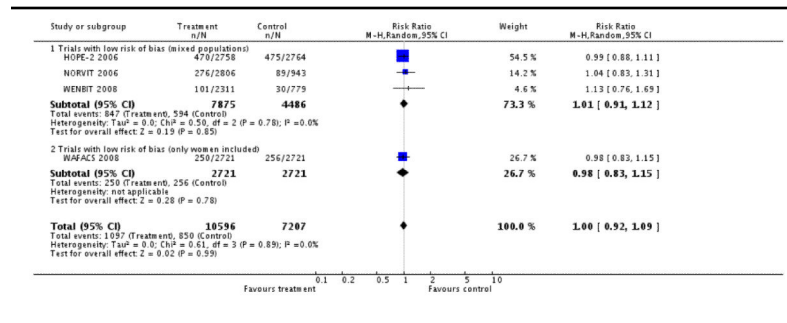
Review: Homocysteine lowering interventions for preventing cardiovascular events

Comparison: 2 Homocysteine-lowering treatment versus other (Sensitivity analysis)

Outcome: 3 First unstable angina pectoris episode requiring hospitalization

Analysis 2.4. Comparison 2 Homocysteine-lowering treatment versus other (Sensitivity analysis), Outcome 4 Death by any cause

Review: Homocysteine lowering interventions for preventing cardiovascular events

Comparison: 2 Homocysteine-lowering treatment versus other (Sensitivity analysis)

Outcome: 4 Death by any cause

Study or subgroup	Treatment n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Trials with low risk of bias (mixed populations)					
HOPE-2 2006	470/2758	475/2764		54.5 %	0.99 [0.88, 1.11]
NORVIT 2006	276/2806	89/943		14.2 %	1.04 [0.83, 1.31]
WENBIT 2008	101/2311	30/779		4.6 %	1.13 [0.76, 1.69]
Subtotal (95% CI)	7875	4486		73.3 %	1.01 [0.91, 1.12]

Total events: 847 (Treatment), 594 (Control)
Heterogeneity: Tau² = 0.0; Chi² = 0.50, df = 2 (P = 0.78); I² =0.0%
Test for overall effect: Z = 0.19 (P = 0.85)

2 Trials with low risk of bias (only women included)					
WAFACS 2008	250/2721	256/2721		26.7 %	0.98 [0.83, 1.15]
Subtotal (95% CI)	2721	2721		26.7 %	0.98 [0.83, 1.15]

Total events: 250 (Treatment), 256 (Control)
Heterogeneity: not applicable
Test for overall effect: Z = 0.28 (P = 0.78)

Total (95% CI)	10596	7207		100.0 %	1.00 [0.92, 1.09]

Total events: 1097 (Treatment), 850 (Control)
Heterogeneity: Tau² = 0.0; Chi² = 0.61, df = 3 (P = 0.89); I² =0.0%
Test for overall effect: Z = 0.02 (P = 0.99)

0.1 0.2 0.5 1 2 5 10
Favours treatment   Favours control

HISTORY

Protocol first published: Issue 3, 2007

Review first published: Issue 4, 2009

Appendix 1. Search strategies

CENTRAL (*The Cochrane Library* 2008, Issue 3)

#1 MeSH descriptor Vitamin B Complex explode all trees

#2 "vitamin b*"

#3 folic next acid in Title, Abstract or Keywords

#4 folate* in Title, Abstract or Keywords

#5 (homocyst* near/6 lower*)

#6 (homocyst* near/6 reduc*)

#7 pyridoxin*

#8 cobalamin*

#9 cyanocobalamin*

#10 pyridoxol*

#11 MeSH descriptor Vitamins this term only

#12 (vitamin* and homocyst*)

#13 multivitamin*

#14 (#1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13)

#15 MeSH descriptor Cardiovascular Diseases this term only

#16 MeSH descriptor Myocardial Ischemia explode all trees

#17 MeSH descriptor Brain Ischemia explode all trees

#18 MeSH descriptor Cerebrovascular Disorders this term only

#19 (coronary near/6 disease)

#20 angina

#21 myocardial next infarct*

#22 heart next infarct*

#23 (stroke or strokes)

#24 (cerebr* near/6 accident*)

#25 (cerebr* near/6 infarct*)

#26 (brain near/6 infarct*)

#27 apoplexy

#28 cardiovascular next disease*

#29 (cardiovascular near/6 event*)

#30 MeSH descriptor Hyperhomocysteinemia explode all trees

#31 hyperhomocyst*

#32 cva

#33 (#15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25)

#34 (#26 or #27 or #28 or #29 or #30 or #31 or #32)

#35 (#33 or #34)

#36 (#14 and #35)

Lilacs (accessed through Biblioteca Virtual em Saúde)

((Pt ENSAYO CONTROLADO ALEATORIO OR Pt ENSAY CLINICO
CONTROLADO OR Mh ENSAYOS CONTROLADOS ALEATORIOS OR Mh
DISTRIBUCIÓN ALEATORIA OR Mh METODO DOBLE CIEGO OR Mh METODO
SIMPLECIEGO OR Pt ESTUDIO MULTICÉNTRICO) or ((tw ensaio or tw ensayo or tw
trial) and (tw azar or tw acaso or tw placebo or tw control$ or tw aleat$ or tw random$ or

(tw duplo and tw cego) or (tw doble and tw ciego) or (tw double and tw blind)) and tw clinic $)) AND NOT ((Ct ANIMALES OR Mh ANIMALES OR Ct CONEJOS OR Ct RATÓN OR MH Ratas OR MH Primates OR MH Perros OR MH Conejos OR MH Porcinos) AND NOT (Ct HUMANO AND Ct ANIMALES)) [Palavras] and MH Vitamina B 12 OR Cobamidas OR Hidroxocobalamina OR Complejo Vitamínico B OR Ácido Fólico OR Ácidos Pteroilpoliglutámicos OR Tetrahidrofolatos OR Formiltetrahidrofolatos OR Vitamina B 6 OR Piridoxal OR Fosfato de Piridoxal OR Piridoxamina OR Piridoxina OR Homocisteína OR Vitaminas or TW vitamin$ or tw cobalamin$ or tw cianocobalamin$ or tw cyanocobalam$ or tw cobamid$ or tw hidroxocobalam$ or tw Hydroxocobalam$ or ((tw complejo or tw complex$) and tw vitamin$ and tw b) or (tw acid$ and (tw folic$ or tw ptero $)) or tw Tetrahidrofolatos or tw Formiltetrahidrofolatos or (tw vitamin$ or (tw b or tw b6 or tw b12)) or tw Piridoxal or tw Pyridoxal or ((tw Fosfat$ or tw phosphate$) and (tw Piridoxal or tw pyridoxal)) or tw Piridox$ or tw Pyridox$ or tw Homocisteína or tw Homocysteine) AND (MH Enfermedades Cardiovasculares or Isquemia Miocárdica or Ex C14.280.647$ or Isquemia Encefálica or Ex C10.228.140.300.150$ or Trastornos Cerebrovasculares or hiperhomocisteinemia or Accidente Cerebrovascular or ((tw apoplexia or tw derrame or tw trastorno$ or tw accident$ or tw acidente or tw stroke$ or tw disease$ or tw enfermedad$ or tw doenca$ or tw event$ or tw infart$ or tw isquemia or tw disorder$) and (tw miocardio or tw myocard$ or tw cerebr$ or tw cardiovascul$ or tw heart or tw cardiovascul$ or tw encefal $)) or tw hyperhomocyst$ or tw hiperhomocisteinemia) [Palavras]

MEDLINE (accessed through Ovid)

1. exp Vitamin B Complex/ (104482)

2. vitamin b.tw. (4924)

3. folic acid.tw. (9923)

4. folate$.tw. (13932)

5. ((homocystein$ or homocystin$) adj3 (low$ or reduc$)).tw. (1436)

6. pyridoxin$.tw. (3952)

7. cobalamin$.tw. (2802)

8. cyanocobalamin$.tw. (1001)

9. pyridoxol$.tw. (85)

10. Vitamins/ (14164)

11. or/1-10 (126718)

12. Cardiovascular Diseases/ (64258)

13. exp Myocardial Ischemia/ (291587)

14. exp Brain Ischemia/ (62826)

15. Cerebrovascular Disorders/ (41502)

16. (coronary adj3 disease$).tw. (79742)

17. angina.tw. (36699)

18. myocardial infarct$.tw. (107782)

19. heart infarct$.tw. (708)

20. heart attack$.tw. (2655)

21. (stroke or strokes).tw. (89789)

22. (cerebr$ adj3 (accident$ or infarct$)).tw. (15976)

23. (brain adj3 infarct$).tw. (3151)

24. apoplexy.tw. (1922)

25. (cardiovascular adj2 (disease$ or event$)).tw. (60351)

26. Hyperhomocysteinemia/ (2497)

27. hyperhomocyst?in?emi$.tw. (3501)

28. or/12-27 (553136)

29. 11 and 28 (6360)

30. randomized controlled trial.pt. (264114)

31. controlled clinical trial.pt. (79970)

32. Randomized controlled trials/ (56782)

33. random allocation/ (62626)

34. double blind method/ (100106)

35. single-blind method/ (12467)

36. or/30-35 (445752)

37. exp animal/ not humans/ (3351598)

38. 36 not 37 (417326)

39. clinical trial.pt. (457582)

40. exp Clinical Trials as Topic/ (211137)

41. (clin$ adj25 trial$).ti,ab. (151447)

42. ((singl$ or doubl$ or trebl$ or tripl$) adj (blind$ or mask$)).ti,ab. (96859)

43. placebos/ (28068)

44. placebo$.ti,ab. (113353)

45. random$.ti,ab. (424142)

46. research design/ (54217)

47. or/39-46 (942009)

48. 47 not 37 (874873)

49. 38 or 48 (899775)

50. 49 and 29 (1537)

EMBASE (accessed through Ovid)

1. exp Vitamin B Group/ (75410)

2. vitamin b.tw. (1818)

3. folic acid.tw. (6853)

4. folate$.tw. (11356)

5. ((homocystein$ or homocystin$) adj3 (low$ or reduc$)).tw. (1389)

6. pyridoxin$.tw. (2405)

7. cobalamin$.tw. (2261)

8. cyanocobalamin$.tw. (597)

9. pyridoxol$.tw. (41)

10. Vitamins/ (9853)

11. or/1-10 (88156)

12. Cardiovascular Diseases/ (69944)

13. exp ischemic heart disease/ (223039)

14. exp Coronary Artery Disease/ (81147)

15. exp Brain Ischemia/ (42318)

16. cerebrovascular disease/ (15529)

17. stroke/ (63052)

18. cerebrovascular accident/ (21560)

19. (coronary adj3 disease$).tw. (68830)

20. angina.tw. (29346)

21. myocardial infarct$.tw. (82436)

22. heart infarct$.tw. (773)

23. heart attack$.tw. (1913)

24. (stroke or strokes).tw. (81585)

25. (cerebr$ adj3 (accident$ or infarct$)).tw. (13910)

26. (brain adj3 infarct$).tw. (2838)

27. apoplexy.tw. (970)

28. (cardiovascular adj2 (disease$ or event$)).tw. (54102)

29. Hyperhomocysteinemia/ (4343)

30. hyperhomocyst?in?emi$.tw. (3426)

31. or/12-30 (467011)

32. 11 and 31 (7063)

33. controlled clinical trial/ (50501)

34. random$.tw. (378547)

35. randomized controlled trial/ (161864)

36. follow-up.tw. (340013)

37. double blind procedure/ (70310)

38. placebo$.tw. (107162)

39. placebo/ (117448)

40. factorial$.ti,ab. (7812)

41. (crossover$ or cross-over$).ti,ab. (38508)

42. (double$ adj blind$).ti,ab. (83051)

43. (singl$ adj blind$).ti,ab. (7269)

44. assign$.ti,ab. (104756)

45. allocat$.ti,ab. (33066)

46. volunteer$.ti,ab. (96369)

47. Crossover Procedure/ (20586)

48. Single Blind Procedure/ (7760)

49. or/33-48 (983879)

50. 32 and 49 (1637)

Allied and Complementary Medicine - AMED (accessed through Ovid)

1. exp Vitamin B Complex/ (216)

2. vitamin b.tw. (181)

3. folic acid.tw. (102)

4. folate$.tw. (62)

5. ((homocystein$ or homocystin$) adj3 (low$ or reduc$)).tw. (18)

6. pyridoxin$.tw. (39)

7. cobalamin$.tw. (12)

8. cyanocobalamin$.tw. (3)

9. pyridoxol$.tw. (0)

10. Vitamins/ (771)

11. or/1-10 (1061)

12. (coronary adj3 disease$).tw. (863)

13. angina.tw. (226)

14. myocardial infarct$.tw. (559)

15. heart infarct$.tw. (0)

16. heart attack$.tw. (47)

17. (stroke or strokes).tw. (4972)

18. (cerebr$ adj3 (accident$ or infarct$)).tw. (1762)

19. (brain adj3 infarct$).tw. (39)

20. apoplexy.tw. (59)

21. (cardiovascular adj2 (disease$ or event$)).tw. (1148)

22. hyperhomocyst?in?emi$.tw. (13)

23. exp cardiovascular disease/ (10808)

24. or/12-23 (12172)

25. 11 and 24 (95)

26. randomized controlled trial.pt. (1200)

27. randomised controlled trial.pt. (67)

28. random$.tw. (9697)

29. factorial$.tw. (223)

30. (crossover$ or cross over$).tw. (617)

31. placebo$.tw. (2128)

32. (double$ adj blind).tw. (1270)

33. (singl$ adj blind$).tw. (361)

34. assign$.tw. (2815)

35. allocat$.tw. (1211)

36. volunteer$.tw. (2403)

37. double blind method/ (386)

38. random allocation/ (288)

39. exp clinical trials/ (2938)

40. or/26-39 (15394)

41. 25 and 40 (10)

ISI Web of Science

11 TS=(#10 and (random* or blind* or placebo* or comparative or comparison or prospective or controlled or trial or evaluation or rct))

10 #7 or #8 or #9

9 TS=(#6 and ("cerebrovascular accident*" or hyperhomocyst*))

8 TS=(#6 and (angina or stroke or strokes or cva or infarction*))

7 TS=(#6 and (cardiovascular or myocardial or coronary or cardiac or "heart disease*"))

6 #1 or #2 or #3 or #4 or #5

5 TS=(homocyst* same (lower* or reduc*))

4 TS=(vitamin* and homocyst*)

3 TS=folate*

2 TS="vitamin B"

1 TS=(pyridoxin* or cobalamin* or cyanocobalamin* or pyridoxol* or "folic acid")

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* *Indicates the major publication for the study*

PLAIN LANGUAGE SUMMARY

B-complex vitamin therapy for preventing cardiovascular events

Cardiovascular disease is the number one cause of death worldwide. The most common causes of its morbidity and mortality are ischaemic heart disease, stroke and congestive heart failure. Many people with cardiovascular diseases may be asymptomatic, and might have high risk for developing a myocardial infarction, angina pectoris, stroke (ischaemic, haemorrhagic or both). 'Emergent or new risk factors' for cardiovascular disease have been recently added to the established risk factors (diabetes mellitus, high blood pressure, active smoker, adverse blood lipid profile). One of these risk factors is an elevated circulating total homocysteine (tHcy) levels. Homocysteine is an amino acid, and its levels in blood are influenced by blood levels of B-complex vitamins: cyanocobalamin (B12), folic acid (B9) and pyridoxine (B6). High tHcy levels are associated with an increased risk for atherosclerotic diseases. Hence, it has been suggested that B vitamins supplementation might reduce the risk of myocardial infarction, stroke, angina pectoris. Preventive strategies might include healthy people with low or high risk for developing cardiovascular disease (primary prevention) and people with an established cardiovascular disease (secondary prevention). In this review we included eight randomised controlled trials equivalent to 24,210 participants. We found no evidence that homocysteine-lowering interventions, in the form of supplements of vitamins B6, B9 or B12 given alone or in combination, at any dosage compared with placebo or standard care, prevents myocardial infarction, stroke, or reduces total mortality in participants at risk or with established cardiovascular disease.

Blood concentrations of homocysteine at baseline and end follow-up (µmol/L)

| STUDY	YEAR	INTERVENTION						CONTROL	
		Baseline			End follow-up			Baseline	End follow-up
CHAOS-2	2002	11.2 ± 6.9			9.7 ± 5.3			Not reported	Not reported
FOLARDA	2004	Not reported			Not reported			Not reported	Not reported
GOES	2003	12.0 ± 4.8			Not reported			12.2 ± 3.8	Not reported
HOPE-2	2006	12.2			At 2 years: 9.9 (mean) Decreased at 5 years of follow up: 9.7 Difference in the change from baseline between groups at 2 years of follow up: 3.3. Difference in the change from baseline between groups at 5 years of follow-up: 3.2.			12.2	Increase (mean) at 2 years of follow up: 13.2 Increase (mean) at 5 years of follow up: 12.9
NORVIT	2006	I 13.1 ± 5.0	II 12.9 ± 4.3	III 13.3 ± 6.1	I 9.5 ± 3.6	II 9.8 ± 4.0	III 13.3 ± 5.4	13.2 ± 5.2	13.6 ± 6.2
VISP	2004	13.4			Decreased 2.0 at 1 month; 2.2 at 1 year, and 2.3 at 2 years.			13.4	Decreased 0.3 at month Not described information at 1 and 2 years.
WAFACS	2008	Median: 12.1 (interquartile range – IR–: 10.2–15.0)			Median: 9.8 (IR: 7.9 – 12.4).			Median: 12.5 (IR: 9.6 – 15.5)	Median: 11.8 (IR: 9.8 – 14.9)
WENBIT	2008	Not described			Not described			Not described	Not described

Figure 1.
Blood homocysteine levels.
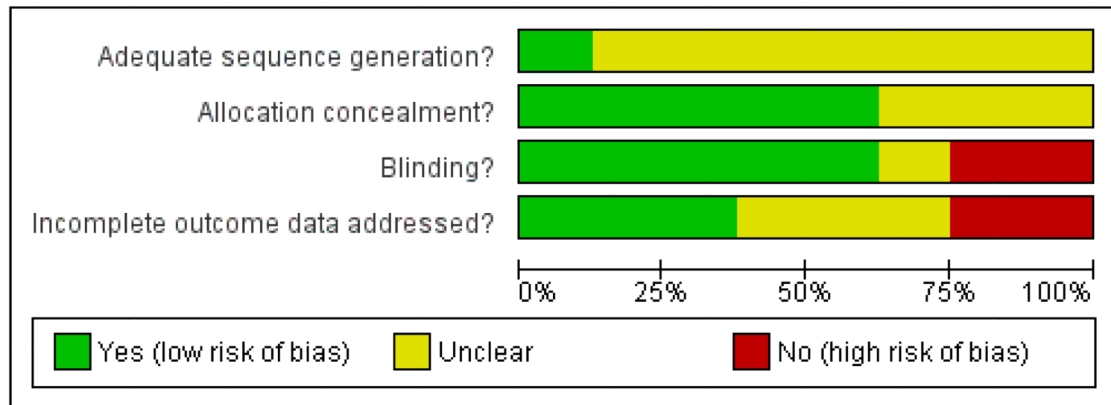
Figure 2.
Methodological quality graph: review authors' judgements about each methodological quality item presented as percentages across all included studies.

Figure 3.
Methodological quality summary: review authors' judgements about each methodological quality item for each included study.

Figure 4.
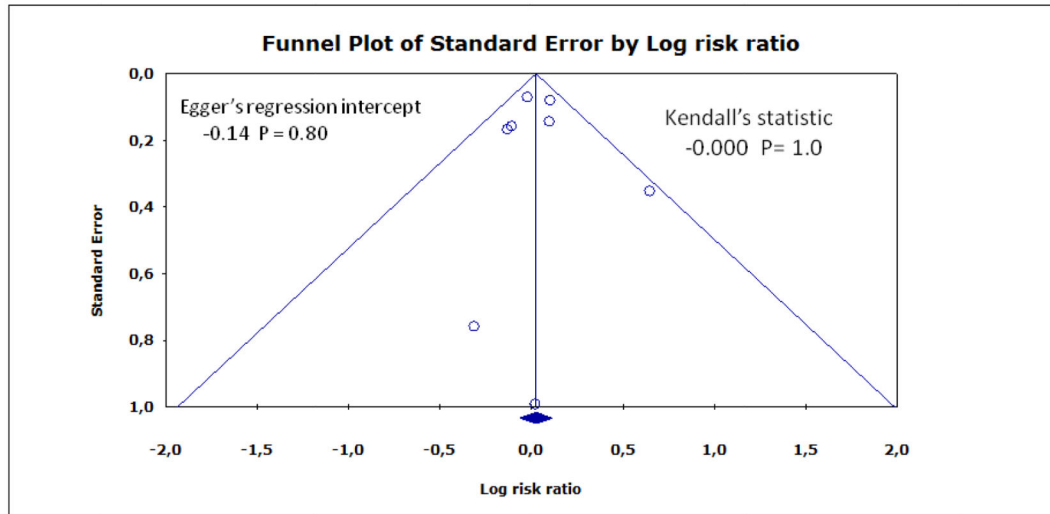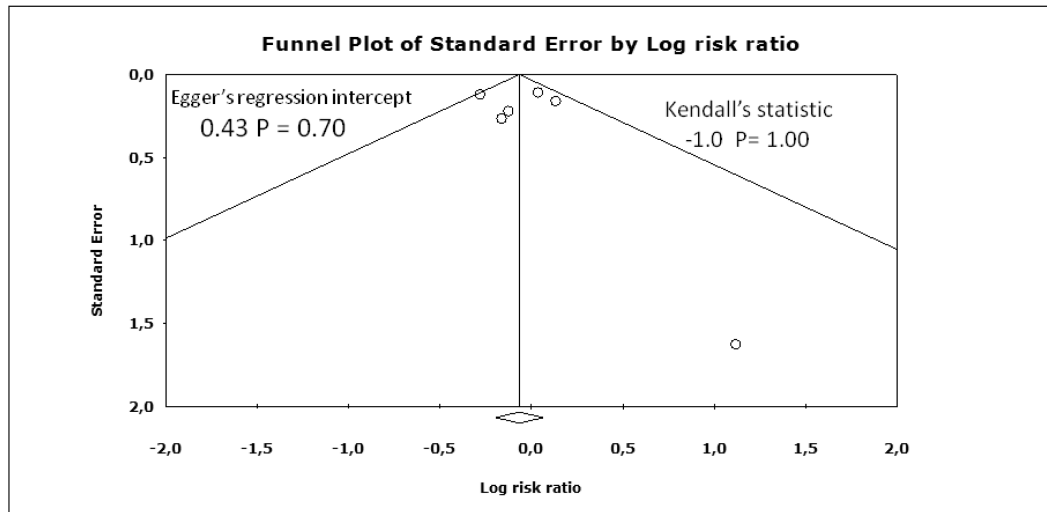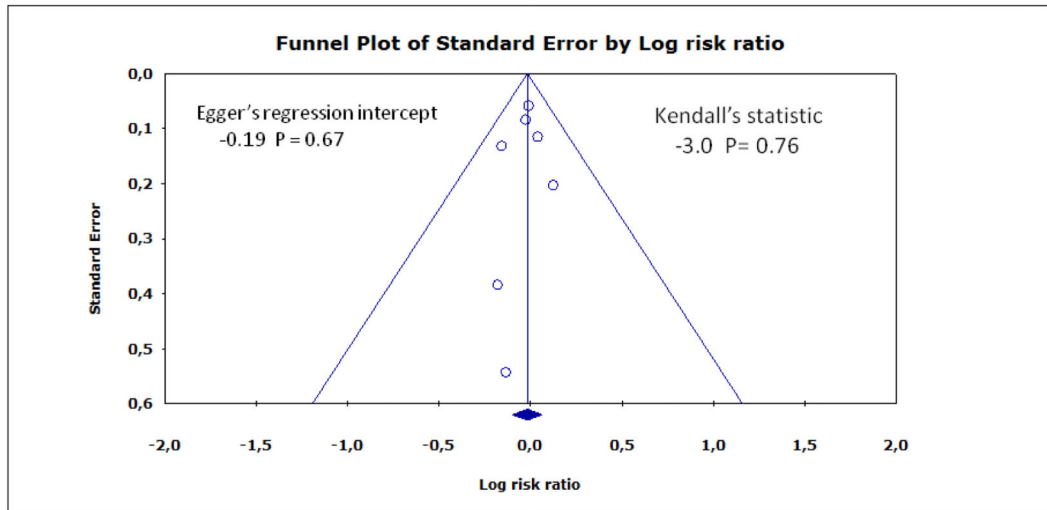Funnel plot for myocardial infarction.

Figure 5.
Funnel plot for stroke

Figure 6.
Funnel plot for death

Study Year	Conclusions of the included randomized controlled trials
CHAOS 2002	"The study *does not support routine use* of folic acid use supplementation in patients with ischemic heart disease and slight elevation of plasma hcy".
FOLARDA 2004	"In this medium-size pilot study, *folic acid did not demonstrate* any beneficial additive effects on cardiovascular mortality or morbidity in post-MI patients with hypercholesterolemia who were treated with statin therapy. Larger trials, possibly targeting at selected populations, must be awaited before definitive conclusions regarding the potentially favourable effects of folic acid supplementation in secondary prevention can be drawn".
GOES 2003	"Within two years, *folic acid does not seem to reduce* clinical end points in patients with stable coronary artery disease (CAD) while on statin treatment. Homocysteine might therefore merely be a modifiable marker of disease. Thus, low-dose folic acid supplementation should be be treated with reservation, until more trial outcomes become available".
HOPE-2 2006	"Supplements combining *folic acid and vitamins B6 and B12 did not reduce* the risk of major cardiovascular events in patients with vascular disease".
NORVIT 2006	"Treatment with *B vitamins did not lower the risk of recurrent cardiovascular disease* after acute myocardial infarction. A harmful effect from combined B vitamin treatment was suggested. Such treatment should therefore not be recommended".
VISP 2004	"In this trial, moderate reduction of total homocysteine after non disabling cerebral infarction *had no effect on vascular outcomes* during the 2 years of follow up. However, the consistent findings of an association of total homocysteine with vascular risk suggests that further exploration of the hypothesis is warranted and longer trials in different populations with elevated total homocysteine may be necessary".
WAFACS 2008	"These data, along with those from previously published randomized trials, *do not support the use of folic acid and vitamin B supplements* as preventive".
WENBIT 2008	"This trial *did not find an effect of treatment with folic acid/vitamin B12 or vitamin B6* on total mortality or cardiovascular events. Our findings do not support the use of B vitamins as secondary prevention in patients with coronary artery disease".

Figure 7.
Conclusions from the included RCT in this Cochrane review

Table 1

Burden of deaths attributable to cardiovascular diseases (%) (From Gaziano 2006)

Region	%
Sub-Saharan Africa, parts of all regions excluding high-income regions	5 to 10
South Asia, southern East Asia and the Pacific, parts of Latin America and the Caribbean	15 to 35
Europe and Central Asia, northern East Asia and the Pacific, Latin America and the Caribbean, Middle East and North Africa, and urban parts of most low-income regions (especially India)	> 50
High-income countries, parts of Latin America and the Caribbean	< 50

Table 2

Composition of intervention and control groups

Study	Year	Folic acid (mg/d)	Vitamin B12 (mg/d)	Vitamin B6 (mg/d)	Control
CHAOS-2	2002	5	None	None	Placebo
FOLARDA	2004	5	None	None	Standard care
GOES	2003	0.5	None	None	Standard care
HOPE-2	2006	2.5	1	50	Placebo
NORVIT	2006	Group A: 0.8; Group B: 0.8; Gruop C: none	Group A: 0.4; Group B: 0.4; Group C: none	Group A: none; Group B: none; Group C: 40	Placebo
VISP	2004	2.5	0.4	25	Folic acid: 0.02; Vit.B12: 0.06; Vita B6: 0.2
WAFACS	2008	2.5	1	50	Placebo
WENBIT	2008	Group A: 0.8; Group B: 0.8; Gruop C: none	Group A: 0.4; Group B: 0.4; Group C: none	Group A: none; Group B: none; Group C: 40	Placebo

Table 3

Components of the composite outcomes

Study	Year	Components
CHAOS-2	2002	Non-fatal myocardial infarction; cardiovascular death; unplanned revascularization
FOLARDA	2004	Cardiovascular death (sudden death; fatal recurrent myocardial infarction; fatal stroke; death from cardiovascular)
GOES	2003	Death from cardiovascular cause; death from non-cardiovascular cause; recurrent myocardial infarction; invasive coronary procedures; stroke; other vascular surgery
HOPE-2	2006	Death from cardiovascular; myocardial infarction; stroke.
NORVIT	2006	Myocardial infarction (fatal and non-fatal); stroke (fatal and non-fatal); sudden death attributed to coronary heart disease
WAFACS	2008	Myocardial infarction; stroke; revascularization; death from cardiovascular cause
WENBIT	2008	All-cause death; nonfatal acute myocardial infarction; acute hospitalisation for unstable angina pectoris; non-fatal thromboembolic stroke

Table 4

Definitions of myocardial infarction (MI), stroke, unstable angina, and death.

Study	Year	MI	Stroke	Unstable angina	Death	Notes
CHAOS-2	2002	Not available.	Not evaluated in this study.	Not evaluated in this study.	Not available.	
FOLARDA	2004	Creatinine kinase elevation at least two times the upper limit of normal and in addition one of the following: On EKG, new pathological Q-waves in at least two contiguous leads or new left bundle branch block or chest pain longer than 30 min	Unequivocal signs of focal or global neurological deficit with sudden onset was present with a duration greater than 24 h and which were judged to be vascular in origin	Recurrence of typical or atypical anginal pain at rest or at effort with the appearance of a ST segment change >0.1mV and/or T wave inversion in at least 2 of 12 leads	Not available.	
GOES	2003	Two out of three criteria should be positive: chest pain lasting _30 min, creatine kinase elevation _2 times the upper limit of normal or new pathological Q waves of 0.04-s duration or 25% of the corresponding R-wave amplitude, both in at least two contiguous leads	Not available.	Not available.	Not available.	
HOPE-2	2006	Two of the following three criteria were met: typical symptoms, increased cardiac-enzyme levels, and diagnostic electrocardiographic changes$	Focal neurologic deficit lasting more than 24 hours. Computed tomography or magnetic resonance imaging was recommended to identify the type of stroke (ischaemic or hemorrhagic). When these tools were not available, the stroke was classified as of uncertain type	Not available.	Cardiovascular causes were unexpected deaths presumed to be due to ischaemic cardiovascular disease and occurring within 24 hours after the onset of symptoms without clinical or postmortem evidence of another cause, deaths from myocardial infarction or stroke within 7 days after the event, deaths associated with cardiovascular interventions within 30 days after cardiovascular surgery or within 7 days after percutaneous interventions, and deaths from congestive heart failure, arrhythmia, pulmonary embolism, or ruptured aortic aneurysm. Deaths from uncertain causes were presumed to be due to cardiovascular causes	$ Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined - a consensus document of the joint European Society of Cardiology/ American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol 2000;36: 959-69. [Erratum, J Am Coll Cardiol 2001;37:973.]: source not available.

Study	Year	MI	Stroke	Unstable angina	Death	Notes
NORVIT	2006	See supplementary appendix: www.nejm.org	See supplementary appendix: www.nejm.org	See supplementary appendix: www.nejm.org	See supplementary appendix: www.nejm.org	Definitions are very longer to resume here.
VISP	2004	New ECG changes including Q waves or marked ST-T changes plus abnormal cardiac enzymes, cardiac symptoms plus abnormal enzymes, or symptoms plus hyperacute ECG changes resolving with thrombolysis	Evidence of sudden onset of focal neurologic deficit lasting at least 24 hours accompanied by an increased NIHSS Score in an area that was previously normal. When the sudden onset of symptoms lasting at least 24 hours was not accompanied by an increased NIHSS Score in an area that was previously normal, then recurrent stroke was diagnosed using cranial CT or MRI evidence of new infarction consistent with the clinical presentation	Not available.	Not available.	
WAFACS	2008	According to World Health Organizations's criteria.	A new neurologic deficit of sudden onset that persisted for more than 24 jours or until death within 24 hours	Not available.	Due to cardiovascular disease was confirmed by examinations of autopsy reports, death certificates, medical records, and information obtained from the next kin or other family members. Death from any cause was confirmed by the end point committee on the basis of a death certificate	
WENBIT	2008	According to The Joint European Society of Cardiology/ American College of Cardiology Committee. Eur Heart J. 2000;21:1502-13	According to Cannon CP, Battler A, Brindis RG, Cox JL, Ellis SG, Every NR et al. A report of the American College of Cardiology Task Force on Clinical Data Standards (Acute Coronary Syndromes Writing Committee). J Am Coll Cardiol. 2001;38: 2114-30	According to Cannon CP, Battler A, Brindis RG, Cox JL, Ellis SG, Every NR et al. A report of the American College of Cardiology Task Force on Clinical Data Standards (Acute Coronary Syndromes Writing Committee). J Am Coll Cardiol. 2001; 38: 2114-30	If death occurred within 28 days after the onset of an event, the event was classified as fatal.	

Table 5

Secondary publication

Included Study	Reference	Objective	Data added addendum
GOES 2003	Liem A, Reynierse-Buitenwerf GH, Zwinderman AH, Jukema JW, van Veldhuisen DJ. Secondary prevention with folic acid: results of the Goes extension study. Heart 2005;91:1213-4	To report the results of the study with a mean (SD) follow up of 42 (10) months.	Hcy has to be interpreted as a modifiable risk marker without apparent clinically salutary effects on hard clinical outcomes in patients with stable coronary artery disease
VISP 2004	Spence JD, Bang H, Chambless LE, Stampfer MJ. Vitamin Intervention For Stroke Prevention trial: an efficacy analysis. Stroke 2005;36:2404-09	This RCT was an efficacy analysis limited to patients most likely to benefit from the treatment, based on hypotheses arising from evidence developed since VISP was initiated	In the era of folate fortification, B12 plays a key role in vitamin therapy for total homocysteine. Higher doses of B12, and other treatments to lower total homocysteine may be needed for some patients
WAFACS 2008	Redbeg RF, Block PC. A randomised trial of folic acid and B-vitamins in the secondary prevention of cardiovascular events in women: Results from the Women's Antioxidant and Folic Acid Cardiovascular Study (WAFACS). ACC Cardiosource Review Journal. 2006;16(7): 52-58	This RCT is a preliminary report of WAFAC study.	