# Homocysteine-lowering interventions for preventing cardiovascular events (Review)

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[Intervention Review]

# Homocysteine-lowering interventions for preventing cardiovascular events

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**Editorial group:** Cochrane Heart Group.
**Publication status and date:** New search for studies and content updated (no change to conclusions), published in Issue 1, 2015.
**Review content assessed as up-to-date:** 12 February 2014.

**Citation:** Martí-Carvajal AJ, Solà I, Lathyris D. Homocysteine-lowering interventions for preventing cardiovascular events. *Cochrane Database of Systematic Reviews* 2015, Issue 1. Art. No.: CD006612. DOI: 10.1002/14651858.CD006612.pub4.



## ABSTRACT

### Background

Cardiovascular disease, which includes coronary artery disease, stroke and congestive heart failure, is a leading cause of death worldwide. Homocysteine is an amino acid with biological functions in methionine metabolism. A postulated risk factor is an elevated circulating total homocysteine level, which is associated with cardiovascular events. The impact of homocysteine-lowering interventions, given to patients in the form of vitamins B6, B9 or B12 supplements, on cardiovascular events. This is an update of a review previously published in 2009 and 2013.

### Objectives

To determine whether homocysteine-lowering interventions, provided in patients with and without pre-existing cardiovascular disease are effective in preventing cardiovascular events, as well as all-cause mortality and evaluate their safety.

### Search methods

We searched the Cochrane Central Register of Controlled Trials (CENTRAL 2014, Issue 1), MEDLINE (1950 to January week 5 2014), EMBASE (1980 to 2014 week 6) and LILACS (1986 to February 2014). We also searched Web of Science (1970 to 7 February 2014). We handsearched 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 controlled trials assessing the effects of homocysteine-lowering interventions for preventing cardiovascular events with a follow-up period of one 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 performed study selection, 'Risk of bias' assessment and data extraction in duplicate. We estimated risk ratios (RR) for dichotomous outcomes. We measured statistical heterogeneity using the $I^2$ statistic. We used a random-effects model.

**Main results**

In this second updated Cochrane Review, we identified no new randomised controlled trials. Therefore, this new version includes 12 randomised controlled trials involving 47,429 participants. In general terms, 75% (9/12) trials had a low risk of bias. Homocysteine-lowering interventions compared with placebo did not significantly affect non-fatal or fatal myocardial infarction (1743/23,590 (7.38%) versus 1247/20,190 (6.17%); RR 1.02, 95% confidence interval (CI) 0.95 to 1.10, $I^2$ = 0%, high quality evidence), stroke (968/22,348 (4.33%) versus 974/18,957 (5.13%); RR 0.91, 95% CI 0.82 to 1.0, $I^2$ = 11%, high quality evidence) or death from any cause (2784/22,648 (12.29%) versus 2502/19,250 (10.64%); RR 1.01, 95% CI 0.96 to 1.07, $I^2$ = 6%, high quality evidence). Homocysteine-lowering interventions compared with placebo did not significantly affect serious adverse events (cancer) (1558/18,130 (8.59%) versus 1334/14,739 (9.05%); RR 1.06, 95% CI 0.98 to 1.13; $I^2$ = 0%, high quality evidence).

**Authors' conclusions**

This second update of this Cochrane Review found no evidence to suggest that homocysteine-lowering interventions in the form of supplements of vitamins B6, B9 or B12 given alone or in combination should be used for preventing cardiovascular events. Furthermore, there is no evidence to suggest that homocysteine-lowering interventions are associated with an increased risk of cancer.

## PLAIN LANGUAGE SUMMARY

**Homocysteine-lowering interventions (B-complex vitamin therapy) for preventing cardiovascular events**

**Review question**

We reviewed homocysteine-lowering interventions for preventing cardiovascular events.

**Background**

Cardiovascular disease is the number one cause of death worldwide. The most common causes of cardiovascular disease leading to both morbidity and mortality are ischaemic heart disease, stroke and congestive heart failure. Many people with cardiovascular diseases may be asymptomatic, but may have a high risk of developing myocardial infarction, angina pectoris or stroke (ischaemic, haemorrhagic or both). 'Emergent' or new risk factors for cardiovascular disease have recently been added to the established risk factors (which are diabetes mellitus, high blood pressure, active smoking and an adverse blood lipid profile). One of these risk factors is elevated circulating total homocysteine levels. Homocysteine is an amino acid and its levels in the blood are influenced by blood levels of B-complex vitamins: cyanocobalamin (B12), folic acid (B9) and pyridoxine (B6). High plasma total homocysteine levels are associated with an increased risk of atherosclerotic diseases (where there is a build-up of plaque in the arteries).

**Study characteristics**

In this second update, we included 12 studies involving 47,429 participants living in countries with or without mandatory fortification of foods. These studies compared different regimens of B-complex vitamins (cyanocobalamin (B12), folic acid (B9) and pyridoxine (B6)) with a control or any other comparison. The studies were published between 2002 and 2010.

**Key results**

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, prevent myocardial infarction or stroke, or reduce total mortality in participants at risk of or with established cardiovascular disease. Homocysteine-lowering interventions compared with placebo did not significantly affect serious adverse events (cancer).

**Quality of evidence**

Our confidence in the results of this review is high because the included trials we synthesised were of high quality and conducted with a large number of participants.

## SUMMARY OF FINDINGS FOR THE MAIN COMPARISON [*Explanation*]

**Homocysteine-lowering interventions (folic acid, vitamin B6 and vitamin B12) compared with placebo or standard care for preventing cardiovascular events**

**Patient or population:** Adults at risk of or with established cardiovascular disease
**Settings:** outpatients
**Intervention:** homocysteine-lowering interventions (folic acid, vitamin B6 and vitamin B12)
**Comparison:** placebo or standard care

| Outcomes | Illustrative comparative risks* (95% CI) | | Relative effect (95% CI) | No of participants (studies) | Quality of the evidence (GRADE) | Comments |
|---|---|---|---|---|---|---|
| | Assumed risk | Corresponding risk | | | | |
| | Placebo or standard care | Homocysteine-lowering interventions (folic acid, vitamin B6 and vitamin B12) | | | | |
| **Non-fatal or fatal myocardial infarction**<br>Follow-up: 1 to 7.3 years | **Study population** | | **RR 1.02**<br>(0.95 to 1.1) | 43,290<br>(11 studies) | ⊕⊕⊕⊕<br>**high**[1,2] | |
| | **62 per 1000** | **64 per 1000**<br>(59 to 69) | | | | |
| **Stroke**<br>Follow-up: 1 to 7.3 years | **Study population** | | **RR 0.91**<br>(0.82 to 1.01) | 40,815<br>(9 studies) | ⊕⊕⊕⊕<br>**high**[3,4] | |
| | **52 per 1000** | **47 per 1000**<br>(43 to 52) | | | | |
| **Death from any cause**<br>Follow-up: 1 to 7.3 years | **Study population** | | **RR 1.01**<br>(0.96 to 1.07) | 41,898<br>(10 studies) | ⊕⊕⊕⊕<br>**high**[5,6] | |
| | **130 per 1000** | **131 per 1000**<br>(125 to 139) | | | | |
| **Cancer**<br>Follow-up: 3.4 to 7.3 years | **Study population** | | **RR 1.06**<br>(0.98 to 1.13) | 32,869<br>(7 studies) | ⊕⊕⊕⊕<br>**high**[7,8] | |

| | | | |
|---|---|---|---|
| | **91 per 1000** | **96 per 1000** (89 to 102) | |

*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.
**Very low quality:** We are very uncertain about the estimate.

[1] $I^2 = 0\%$.
[2] 43,290 participants with 2986 events.
[3] $I^2 = 13\%$.
[4] 40,815 participants with 1940 events.
[5] $I^2 = 6\%$.
[6] 41,898 participants with 5286 events.
[7] $I^2 = 0\%$.
[8] 32,869 participants with 2892 events.

# BACKGROUND

## Description of the condition

### The burden of cardiovascular disease

Cardiovascular disease is the number one cause of death worldwide (Jamison 2006; WHO 2002). The term cardiovascular disease 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 cardiovascular disease-related morbidity and mortality are ischaemic heart disease, stroke and congestive heart failure (Gaziano 2006).

The burden of cardiovascular disease 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 (Appendix 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 cardiovascular diseases 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 cardiovascular diseases called "emergent or new risk factors" (NACB 2009).

### Homocysteine as a risk factor for cardiovascular disease

Homocysteine is an amino acid not used in protein synthesis (Blom 2011). Its role is to serve as an intermediate in methionine metabolism (Blom 2011; Hackam 2003; Humphrey 2008; NACB 2009; Selhub 2006), and several observational studies have shown that a raised blood homocysteine level is a risk factor for cardiovascular events (Casas 2005; Danesh 1998; Eikelboom 1999; Ford 2002; Guthikonda 2006; HSC 2002; Jacobsen 2005; Kardesoglu 2011; Refsum 1998; Splaver 2004; Stampfer 1992; Wald 2002; Wang 2005; Williams 2010; Wu 2013). The public significance of raised circulating blood homocysteine levels has been considered (Shelhub 2008). The risk of developing cardiovascular events could be explained by endothelial dysfunction between homocysteine and hydrogen sulfide (Pushpakumar 2014), or by an integration of the roles of homocysteine and folic acid in cardiovascular pathobiology, named methoxistasis (Joseph 2013).

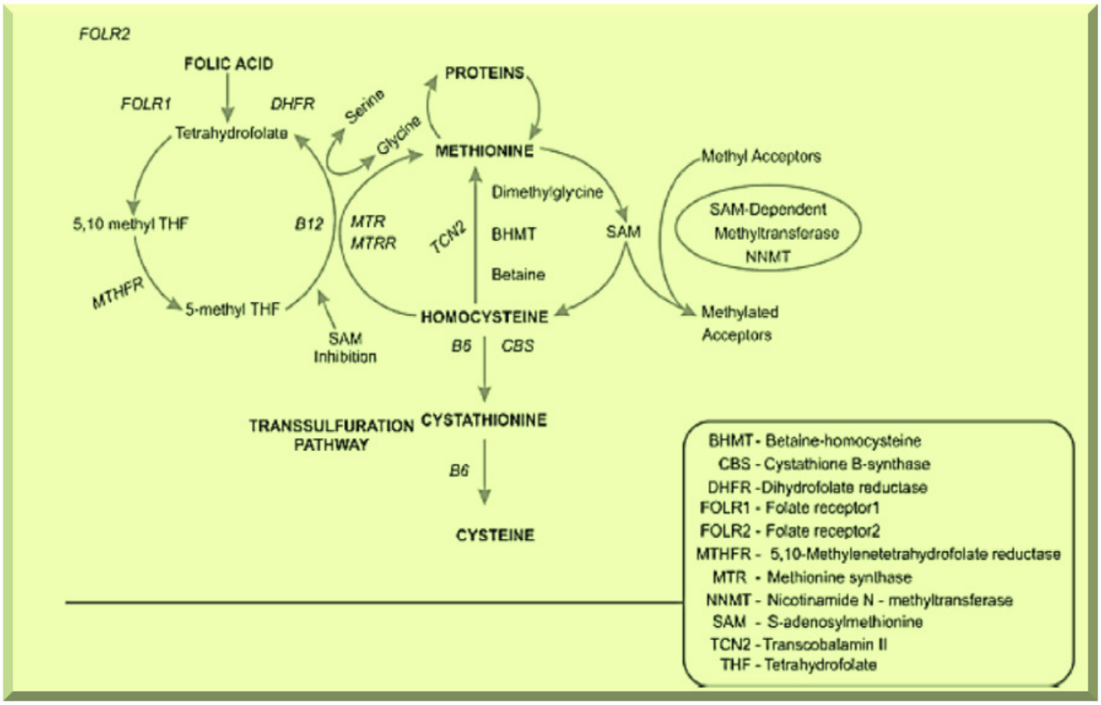
In 1962, it was hypothesised that increased levels of total homocysteine may cause vascular disease: the homocysteine theory of arteriosclerosis (McCully 2005). The pathways through which total homocysteine levels may cause damage to endothelial cells and lead to atherosclerosis have been widely described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

Circulating total homocysteine levels are composed of protein (albumin)-homocysteine mixed disulfide, sulfhydryl form and low molecular weight disulfides (Mudd 2000). The normal levels of total homocysteine are close to 10 µmol/L (Mudd 2000). Hyperhomocysteinaemia is defined as the presence of an abnormally elevated concentration of plasma or serum total homocysteine levels (Mudd 2000). However, there is some controversy about the definition of the degree of hyperhomocysteinaemia. Fasting total homocysteine level concentrations between 12 and 30 µmol/L are termed mild or moderate, while intermediate hyperhomocysteinaemia includes levels between 31 to 100 µmol/L, and severe hyperhomocysteinaemia reflects values above 100 µmol/L (Maron 2006; Maron 2009). In the general population, the prevalence of hyperhomocysteinaemia is between 5% and 10% (Refsum 1998). However, rates may be as high as 30% to 40% in the elderly population (Selhub 1993). According to the results of population-based studies, up to 10% of events due to coronary artery diseases may be attributable to elevated circulating total homocysteine levels (Boushey 1995).

## Description of the intervention

B-complex vitamins, cyanocobalamin (B12) (Fedosov 2012; Herrmann 2012; Kräutler 2012), folic acid (B9) (Crider 2011; Molloy 2012; Ohrvik 2011; Yetley 2011), and pyridoxine (B6) (di Salvo 2011; di Salvo 2012; Friso 2012; Mukherjee 2011), are given as a supplement. Figure 1 shows the role of B-complex vitamins in homocysteine metabolism (Brustolin 2010).

Figure 1. Homocysteine metabolism (Reproduced with Dr Félix TM's permission from Brustolin 2010)




## How the intervention might work

The B-complex vitamins are required for the transformation or excretion, or for both steps, in the total homocysteine level metabolism pathway (Castro 2006; Fowler 2005; Perła-Kaján 2007; Ramakrishnan 2006). Supplementation with B-complex vitamins reduces total homocysteine levels (Clarke 2007; HLTC 2005). There is some ambiguity regarding the function of pyridoxine (vitamin B6). Vitamin B6 supplementation has been shown to lower total homocysteine levels after methionine load, which occurs in an experimental situation and it is, as a result, believed to be a weak determinant of circulating total homocysteine levels. However, at least two studies have shown the contrary (Gori 2007; Sofi 2008). See Figure 1 for details.

## Why it is important to do this review

This is the second update of this Cochrane Review and has been performed to identify the latest evidence, if available.
This review aims to address the following question. What are the benefits and harms of homocysteine-lowering interventions compared with placebo or low-dose vitamins B6, B9 and B12 for preventing cardiovascular events?

# OBJECTIVES

To determine whether homocysteine-lowering interventions, in those patients with and without pre-existing cardiovascular disease:

- are effective for preventing cardiovascular events or all-cause mortality;
- are safe;
- 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 one year or longer.

### Types of participants

Adults (over 18 years) at risk of or with established cardiovascular disease. We excluded studies in patients with end-stage renal disease.

### 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.
We made comparisons with placebo, or with differing regimens of vitamins B6, B9 or B12. When the included population was at risk of cardiovascular disease, we considered combinations of homocysteine-lowering interventions with standard treatment (such as antihypertensives and statins) as long as the same standard treatment was given to the control group.

### Types of outcome measures

#### Primary outcomes

1. Non-fatal or fatal myocardial infarction.
2. Non-fatal or fatal stroke (ischaemic or haemorrhagic stroke).

#### Secondary outcomes

1. First unstable angina pectoris episode requiring hospitalisation.
2. Hospitalisation for heart failure.
3. Death from 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. We considered all other adverse events non-serious.

## Search methods for identification of studies

### Electronic searches

We reran the searches previously run in 2008 (Appendix 2) and 2012 (Appendix 3) on 12 February 2014 (Appendix 4).
We updated the searches of the Cochrane Central Register of Controlled Trials (CENTRAL 2014, Issue 1), MEDLINE OVID (1950 to January week 5 2014), EMBASE OVID (1980 to 2014 week 6) and Web of Science (Thomson Reuters, 1970 to 7 February 2014). The search of LILACS was last run on 2 February 2012.
In the previous version (Marti-Carvajal 2009), we searched Allied and Complementary Medicine - AMED (accessed through Ovid) and the Cochrane Stroke Group Specialised Register.
We used the Cochrane sensitive-maximising RCT filters to search MEDLINE and EMBASE (Lefebvre 2011).
We imposed no language restrictions.

### Searching other resources

We also checked the reference lists of all trials identified.
We also searched the World Health Organization International Clinical Trials Platform search portal (http://apps.who.int/trialsearch).
We contacted authors and researchers to obtain further details for published studies.

## Data collection and analysis

We conducted data collection and analysis of data according to the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011).

### Selection of studies

Three authors (AMC, IS and DS) independently screened the results of the search strategy for potentially relevant trials and independently assessed them for inclusion based on the inclusion criteria.

### Data extraction and management

Two authors (AMC and DS) carried out data extraction using a pre-designed data extraction form that included publication details, patient population, randomisation, allocation concealment, details of blinding measures, description of interventions and results. We resolved discrepancies through discussion. We involved a third author (DEK) to check the data entered into the Review Manager software.

### Assessment of risk of bias in included studies

All review authors independently assessed the risk of bias of the trials according to the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011).
We assessed the following domains, using the following definitions.

#### Generation of the allocation sequence

- Low risk of bias, 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.

- Unclear, if the trial was described as randomised but the method used for the allocation sequence generation was not described.
- High risk of bias, if a system involving dates, names or admittance numbers was used for the allocation of patients. These studies are known as quasi-randomised and we excluded them from the review when assessing beneficial effects.

#### Allocation concealment

- Low risk of bias, if the allocation of patients involved a central independent unit, on-site locked computer, identical-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.
- High risk of bias, if the allocation sequence was known to the investigators who assigned participants or if the study was quasi-randomised. We excluded the latter from the review when assessing beneficial effects.

#### Blinding (or masking)

We assessed each trial (as low, unclear or high risk) with regard to the following levels of blinding.

- Blinding of clinician (person delivering treatment) to treatment allocation.
- Blinding of participant to treatment allocation.
- Blinding of outcome assessor to treatment allocation.

#### Incomplete outcome data

- Low risk of bias, if the numbers and reasons for dropouts and withdrawals in all intervention groups were described or it was specified that there were no dropouts or withdrawals.
- Unclear, if the report gave the impression that there had been no dropouts or withdrawals but this was not specifically stated.
- High risk of bias, if the number or reasons for dropouts and withdrawals were not described.

We further examined the percentage of dropouts overall in each trial and per randomisation arm and we evaluated whether intention-to-treat analysis was performed or could be performed from the published information.

#### Selective outcome reporting

- Low risk of bias, if pre-defined or clinically relevant and reasonably expected outcomes were reported on.
- Unclear, if not all pre-defined or clinically relevant and reasonably expected outcomes were reported on or were not reported on fully, or it was unclear whether data on these outcomes were recorded or not.
- High risk of bias, if one or more clinically relevant and reasonably expected outcomes were not reported on; data on these outcomes were likely to have been recorded.

#### Other bias

- Low risk of bias, the trial appeared to be free of other components that could put it at risk of bias.
- Unclear, the trial may or may not be free of other components that could put it at risk of bias.
- High risk of bias, there were other factors in the trial that could put it at risk of bias.

We considered low risk of bias trials to be those that adequately generated their allocation sequence, had adequate allocation concealment, adequate blinding, adequate handling of incomplete outcome data, were free of selective outcome reporting and were free of other bias.
We considered trials in which we could assess one of domains as high risk of bias or unclear risk of bias as trials with high risk of bias.
Two authors (AMC and IS) assessed the included studies and entered the information into tables; see Characteristics of included studies.

### Measures of treatment effect

We pooled the risk ratios (RR) with 95% confidence interval (CI) for the following binary outcomes: non-fatal or fatal myocardial infarction, non-fatal or fatal stroke (ischaemic or haemorrhagic), first unstable angina pectoris episode requiring hospitalisation, hospitalisation for heart failure, mortality due to any cause and serious or non-serious adverse events as recommended by Higgins 2011.

### Dealing with missing data

For all included trials, we noted the levels of attrition. We contacted the first author of the paper if data were missing. We extracted data on the number of participants by allocated treatment group, irrespective 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-case analysis.

### Assessment of heterogeneity

We quantified statistical heterogeneity using the $I^2$ statistic, which describes the percentage of total variation across studies that is due to heterogeneity rather than sampling error (Higgins 2003). We considered statistical heterogeneity to be present if the $I^2$ value was greater than 50% (Higgins 2011). When significant heterogeneity was detected ($I^2$ > 50%), we attempted to identify the possible causes.

### Assessment of reporting biases

We assessed publication bias for myocardial infarction, stroke and death from any cause using the Comprehensive Meta-Analysis software (CMA 2005).

### Data synthesis

We pooled the results from the trials using the Review Manager software (RevMan 2014). We summarised the findings using a random-effects model.

#### Trial sequential analysis

Meta-analysis of cumulative data may run the risk of random errors ('play of chance') due to sparse data and repetitive analyses of the same data (Brok 2008; Brok 2009; Thorlund 2010; Thorlund 2011; Wetterslev 2008; Wetterslev 2009). In order to assess the risks of random errors in our cumulative meta-analyses, we conducted diversity-adjusted trial sequential analyses based upon the proportion with the outcome in the control group, an a priori set relative risk reduction of 20%, an alpha of 5%, a beta of 20% and the diversity in the meta-analysis (CTU 2011; Thorlund 2009; Thorlund 2011). We conducted sensitivity analysis of the trial sequential analysis to estimate the potential need for further trials.

### Subgroup analysis and investigation of heterogeneity

We performed subgroup analysis according to the type of intervention.

### Sensitivity analysis

We conducted a sensitivity analysis comparing the results using all studies and using only those with low risk of bias.

#### 'Summary of findings' tables

We used the GRADE proposals to assess the quality of the body of evidence associated with the following outcomes: myocardial infarction, stroke, death from any cause and cancer (Guyatt 2011). We constructed Summary of findings for the main comparison (SoF) using the GRADEpro software (GRADEpro 2008). GRADE classifies the quality of a body of evidence based on the extent to which one can be confident that an estimate of effect or association reflects the outcome being assessed (Balshem 2011; Brozek 2011; Guyatt 2011a; Guyatt 2011b; Guyatt 2011c; Guyatt 2011d; Guyatt 2011e; Guyatt 2011f; Guyatt 2011g; Guyatt 2012; Guyatt 2012a).

# RESULTS

## Description of studies

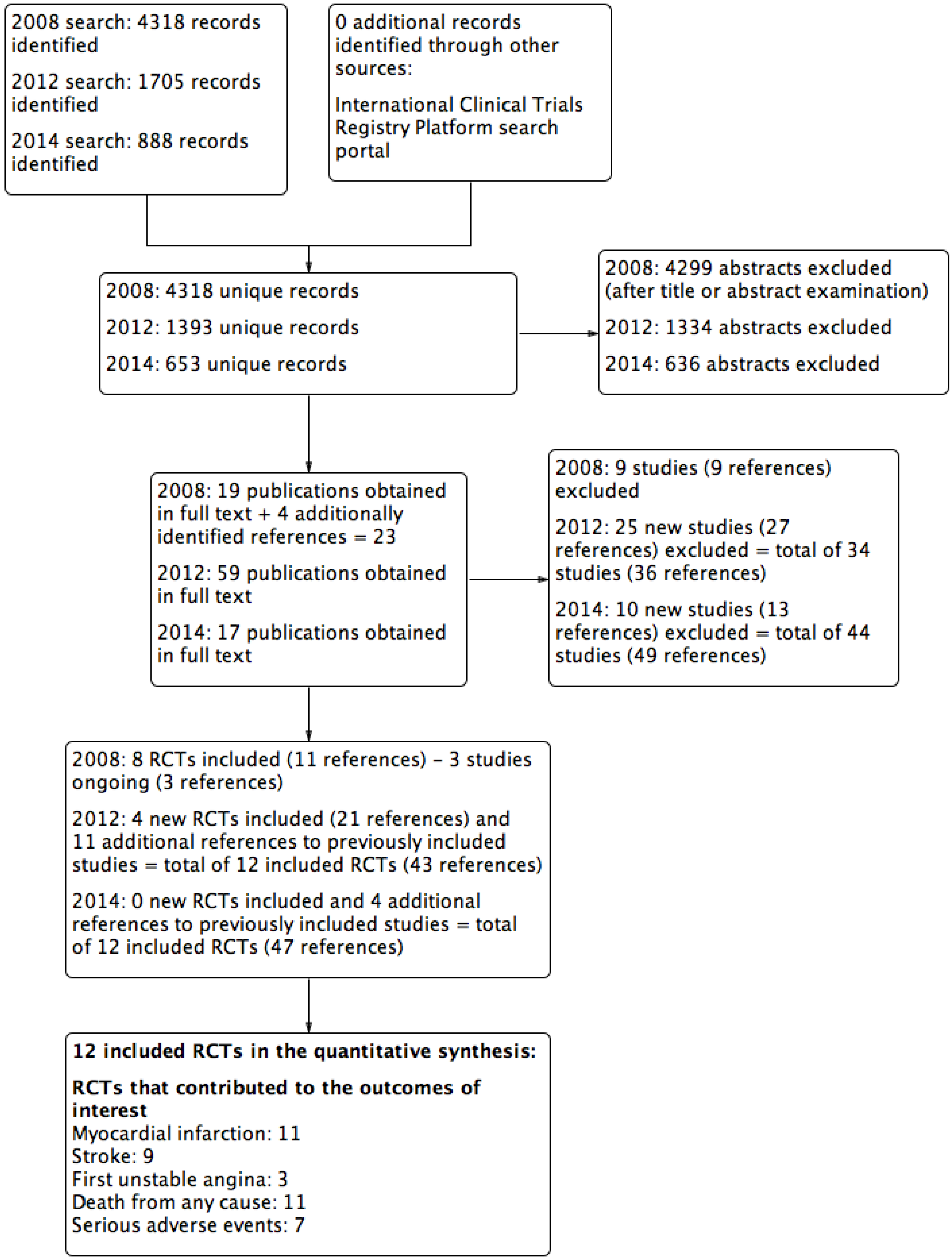
The initial search in 2008 identified 4318 references. From these we excluded 4299 references after examining the title and abstract because they were not relevant. We obtained full reprints of the remaining 19 references for more detailed examination. This led to the identification of four further publications from their reference list. We included eight randomised controlled trials, reported in 11 articles. We subsequently included three identified ongoing studies (three references) in the 2012 update. We excluded nine studies (nine references).

The search in 2012 identified 1705 records, which resulted in 1393 unique references after duplicates were removed. After examining the titles and abstracts we excluded 1334 references. We obtained full reprints of the remaining 59 references for more detailed examination. Four randomised clinical trials, published in 21 references, met the inclusion criteria. We also identified 11 additional references to previously included studies. We excluded 25 new studies (27 references).

The search in February 2014 identified 888 records, which resulted in 653 unique references after duplicates were removed. After examining the titles and abstracts we excluded 636 references. We obtained full reprints of the remaining 17 references for more detailed examination. Ultimately, we were not able to find any new randomised clinical trials.

In total, this updated review includes 12 randomised clinical trials, published between 2002 and 2010, involving 47,429 participants. See Figure 2 for details.

Figure 2. Study flow diagram for this update



These trials are described in Characteristics of included studies. The length of follow-up ranged from one to 7.3 years. The trials varied in size, characteristics of participant populations, duration, drug dosage and experimental design.

### Included studies

Eleven trials were conducted in patients with prior cardiovascular heart disease such as coronary artery disease, myocardial infarction, stable angina, unstable angina, stroke and intermittent claudication (BVAIT 2009; CHAOS 2002; FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VITATOPS 2010; WAFACS 2008; WENBIT 2008); and a further trial explicitly included patients with history of non-disabling cerebral infarction (VISP 2004).

Eleven trials included patients with at least one of the following known cardiovascular risk factors: diabetes mellitus, hypertension elevated total cholesterol, current smoking and low high density lipoprotein (HDL) cholesterol (BVAIT 2009; FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; 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 hyperhomocysteinaemia (total plasma homocysteine > 18 $\mu$mol/L) (FOLARDA 2004).

BVAIT 2009 included patients with hyperhomocysteinaemia without diabetes and cardiovascular disease. HOPE-2 2006 included patients without a history of coronary heart disease (CHD). WAFACS 2008 only included female patients.

Nine trials included more than 1000 patients (CHAOS 2002; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008).

Nine trials were compared with placebo (BVAIT 2009; CHAOS 2002; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VITATOPS 2010; WAFACS 2008; WENBIT 2008), and two with standard care (FOLARDA 2004; GOES 2003), while one trial was a randomised controlled trial (VISP 2004), which compared doses of homocysteine-lowering interventions.

The intervention assessed by most of the trials was a combination of vitamins B6, B9 and B12 (nine trials; BVAIT 2009; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008). Three trials only included vitamin B9 as intervention (CHAOS 2002; FOLARDA 2004; GOES 2003). SU.FOL.OM3 2010 used 5-methyltetrahydrofolate instead of folic acid.

FOLARDA 2004, GOES 2003, HOPE-2 2006, NORVIT 2006, SEARCH 2010, WAFACS 2008 and WENBIT 2008 described lipid-lowering drugs used as concomitant medication. SU.FOL.OM3 2010 reported omega 3 polyunsaturated fatty acids use as concomitant medication.

Three trials, BVAIT 2009, VISP 2004 and 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). Two trials were performed in a mixed population (HOPE-2 2006; VITATOPS 2010), and five were carried out in non-fortified population (CHAOS 2002; FOLARDA 2004; GOES 2003; NORVIT 2006; WENBIT 2008). It was unclear for SEARCH 2010 and SU.FOL.OM3 2010.

Ten trials used composite outcomes in their analyses (CHAOS 2002; FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VITATOPS 2010; WAFACS 2008; WENBIT 2008). Four trials included revascularisation or other vascular procedures (CHAOS 2002; GOES 2003; WAFACS 2008; WENBIT 2008). Eleven trials had stroke as the endpoint (BVAIT 2009; FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008). All trials assessed the impact of the intervention on myocardial infarction rates. None of the trials included pectoris angina as a component of composite outcomes.

Eleven studies reported the sample size calculation (BVAIT 2009; FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008). The trials used 80% or 90% power to detect between 20% and 50% reduction in endpoints.

Blood concentrations of total homocysteine blood levels at baseline were reported in 11 trials (BVAIT 2009; CHAOS 2002; GOES 2003; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008). Five trials reported the total homocysteine blood levels at the end follow-up (CHAOS 2002; HOPE-2 2006; NORVIT 2006; VISP 2004; WAFACS 2008). WENBIT 2008 described total homocysteine blood levels during the intervention: 10.8 (standard deviation (SD) 4.5) $\mu$mol/L at baseline versus to 7.6 (SD 2.2) $\mu$mol/L after one year of the intervention (B9 and B12 groups). There were no changes in the B6 and placebo groups. CHAOS 2002 did not report total homocysteine blood levels at baseline and end of follow-up in the control arm. GOES 2003 reported total homocysteine blood levels at baseline and at the end follow-up only for the intervention arm and not for the control. FOLARDA 2004 did not measure the circulating total homocysteine blood levels in either group.

Definitions used for defining myocardial infarction, stroke, unstable angina and death (any) are described in Appendix 5.

We identified several duplicate publications associated with eight randomised clinical trials (GOES 2003; HOPE-2 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008).

### Excluded studies

We excluded 44 studies in this review (25 in the prior versions and 19 in this update) by the following reasons: systematic review (Bazzano 2006; Clarke 2010; Holmes 2011; Huang 2012; Huo 2012; Jardine 2012; Ji 2013; Lee 2010; Mei 2010; Miller 2010; Pan 2012; Wang 2007; Wang 2012; Yang 2012; Zhang 2009; Zhang 2013; Zhou 2011), narrative review (Lonn 2007; Manolescu 2010; Méndez-González 2010; Ntaios 2009; Vesin 2007; Wierzbicki 2007), randomised clinical trials with a follow-up of less of one year (Earnest 2012; FINEST 2006; Imasa 2009; Lange 2004; PACIFIC 2002; Swiss 2002; Tighe 2011), observational study (Cui 2010; Mager 2009; Moghaddasi 2010; Rautiainen 2010), and miscellaneous (Deshmukh 2010; Durga 2011; Ebbing 2009; Ebbing 2009a; Green 2010; Ntaios 2010; Sharifi 2010; Shidfar 2009; Sudchada 2012; Zappacosta 2013). See table of Characteristics of excluded studies for details.

## Risk of bias in included studies

The risk of bias in the included trials is summarised in Figure 3 and Figure 4, and detailed in the Characteristics of included studies tables.

**Figure 3. Methodological quality graph: review authors' judgements about each methodological quality item presented as percentages across all included studies**

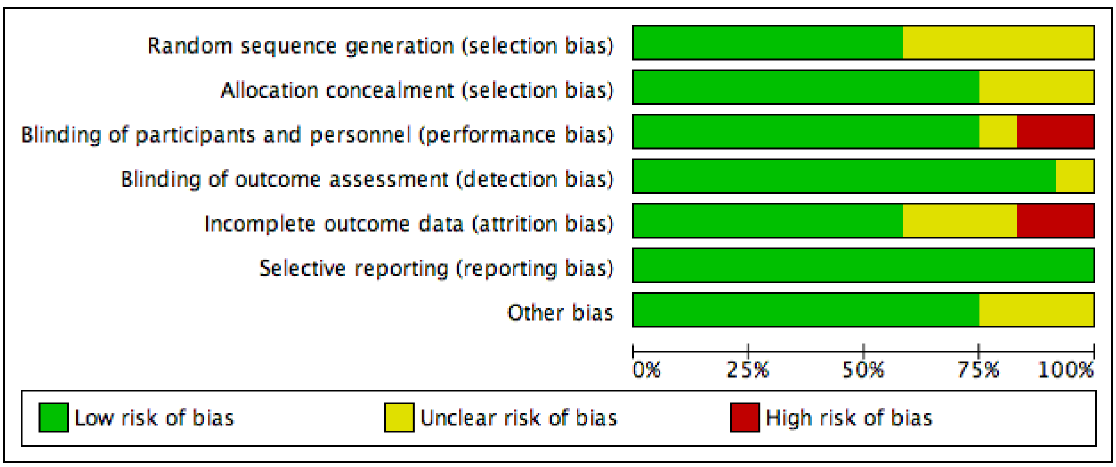
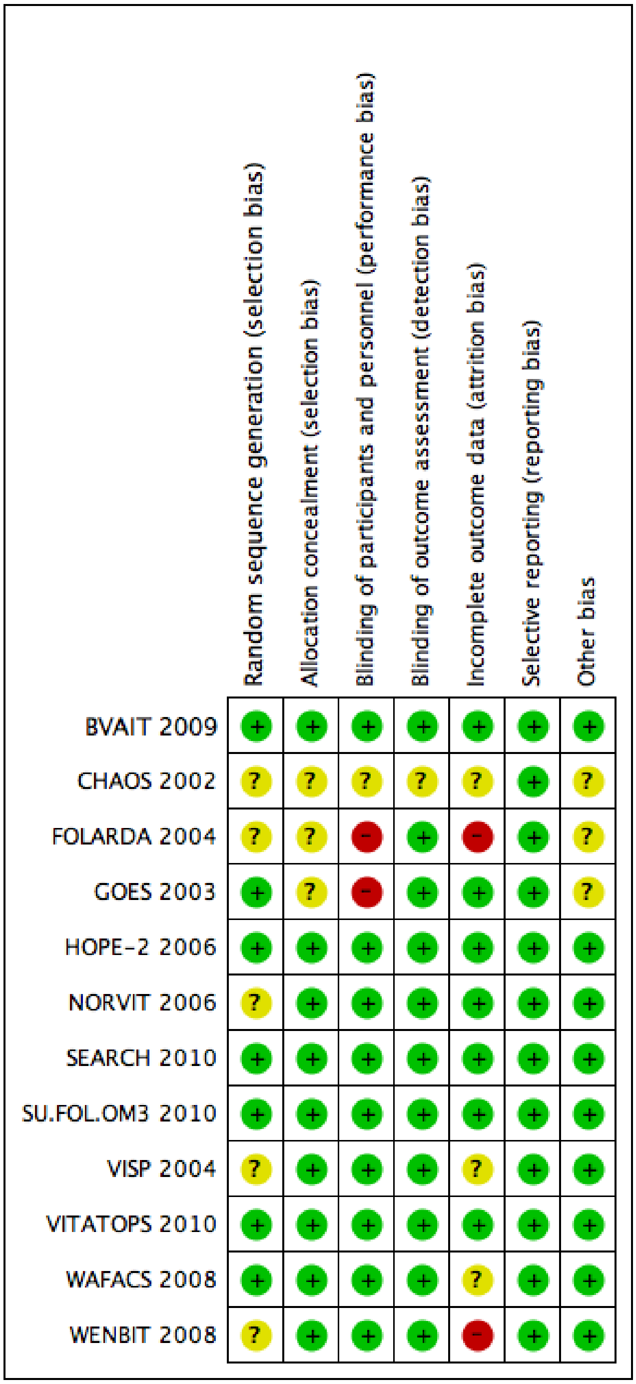


**Figure 4. Methodological quality summary: review authors' judgements about each methodological quality item for each included study**



### Allocation

#### Random sequence generation

The risk of bias arising from the method of generation of the allocation sequence was low in seven trials (BVAIT 2009; GOES 2003; HOPE-2 2006; SEARCH 2010; SU.FOL.OM3 2010; VITATOPS 2010; WAFACS 2008). Five trials had an unclear risk for this domain (CHAOS 2002; FOLARDA 2004; NORVIT 2006; VISP 2004; WENBIT 2008).

#### Allocation concealment

We rated the risk of bias arising from the method of allocation concealment as low in nine trials (BVAIT 2009; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008). Three trials showed an unclear risk for this domain (CHAOS 2002; FOLARDA 2004; GOES 2003).

### Blinding

We rated the risk of bias arising from lack of blinding of participants and personnel as low in nine trials (BVAIT 2009; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008). The risk of bias from blinding was unclear in one trial (CHAOS 2002). We rated the risk of bias arising from lack of blinding as high in two trials (FOLARDA 2004; GOES 2003).

### Incomplete outcome data

We rated the risk of attrition bias as low in seven trials (BVAIT 2009; GOES 2003; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VITATOPS 2010). We rated the risk of attrition bias as high in two trials (FOLARDA 2004; WENBIT 2008). We rated the risk of bias as unclear in three trials (CHAOS 2002; VISP 2004; WAFACS 2008).

### Selective reporting

All trials had a low risk of bias in this domain.

### Other potential sources of bias

Nine trials had a low risk of bias (BVAIT 2009; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008). Three trials had an unclear risk of bias (CHAOS 2002; FOLARDA 2004; GOES 2003).

## Effects of interventions

See: **Summary of findings for the main comparison** Homocysteine-lowering interventions (folic acid, vitamin B6 and vitamin B12) compared with placebo or standard care for preventing cardiovascular events

Results were based on 47,429 participants (Summary of findings for the main comparison).

### Primary outcomes

#### Non-fatal or fatal myocardial infarction

**Homocysteine-lowering interventions compared with placebo or conventional care**

Meta-analysis of 11 randomised clinical trials did not show a difference in non-fatal or fatal myocardial infarction between homocysteine-lowering interventions and placebo or conventional care groups (1747/23,590 (7.40%) versus 1247/20,190 (6.17%); risk ratio (RR) 1.02, 95% confidence interval (CI) 0.95 to 1.10; P value = 0.58, $I^2$ = 0%; high quality evidence) (CHAOS 2002; FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VITATOPS 2010; WAFACS 2008; WENBIT 2008) (Analysis 1.1). Trial sequential analysis for myocardial infarction suggests that no more trials may be needed to disprove an intervention effect of 20% relative risk reduction. Smaller risk reductions might still require further trials (Figure 5). Figure 6 shows the funnel plot for myocardial infarction.

**Figure 5. Trial sequential analysis on myocardial infarction in 11 trials investigating homocysteine-lowering interventions versus placeboTrial sequential analysis of homocysteine-lowering interventions versus placebo on myocardial infarction based on the diversity-adjusted required information size (DARIS) of 10,888 patients. This DARIS was calculated based upon a proportion of patients with myocardial infarction of 6.17% in the control group; a RRR of 20% in the experimental intervention group; an alpha ($\alpha$) of 5%; a beta ($\beta$) of 20%; and a diversity of 0%. The cumulative Z-curve (blue line) does not cross the conventional alpha of 5%. After the fourth trial, the cumulative Z-curve crosses the trial sequential beta-spending monitoring boundary, showing that the area of futility has been reached. This suggests that no more trials may be needed to disprove an intervention effect of 20% relative risk reduction. Smaller risk reductions might still require further trials.**

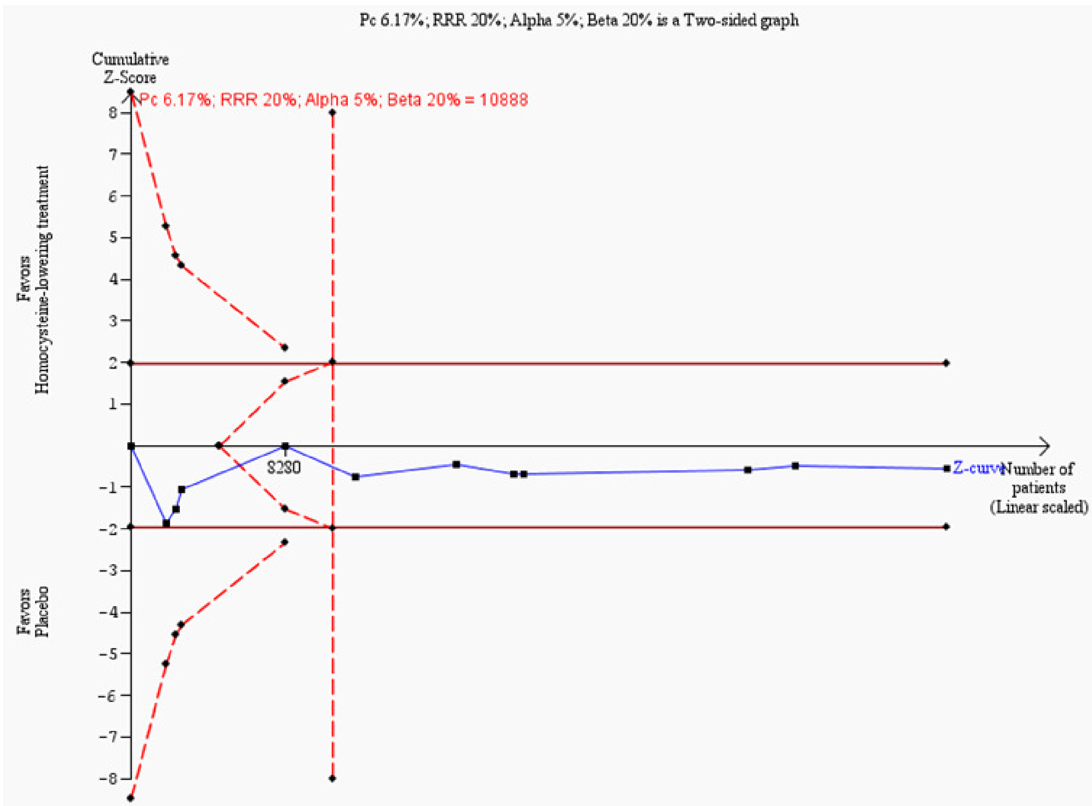
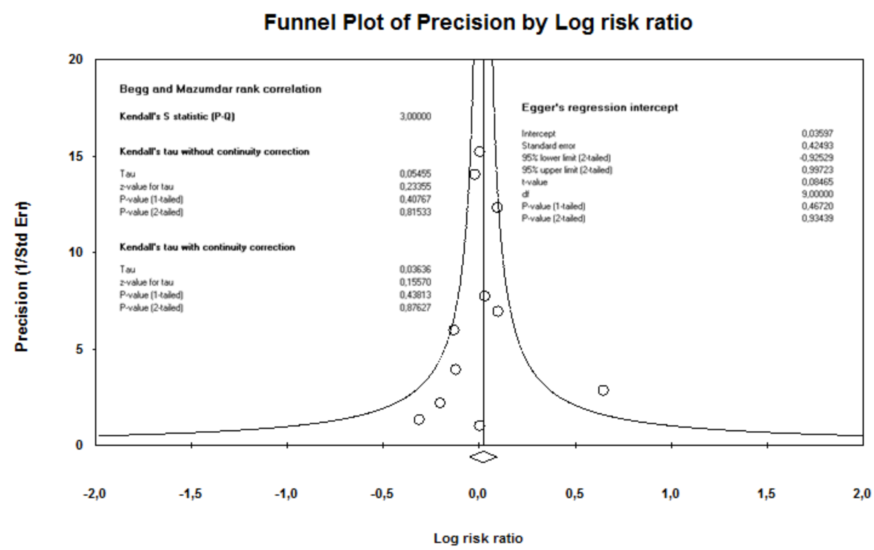


**Figure 6. Funnel plot of data from the meta-analysis of the effects of homocysteine-lowering interventions for preventing myocardial infarctionThe circles show the point estimates of the included randomised clinical trials. The pattern of distribution resembles an inverted funnel. Larger trials are upper and closer to the pooled estimate. The effect sizes of the smaller studies are more or less symmetrically distributed around the pooled estimate. This figure shows a low risk of publication bias.**




**Homocysteine-lowering interventions (high-dose) compared with homocysteine-lowering interventions (low-dose)**

One trial found no significant difference in non-fatal or fatal myocardial infarction between intervention and control groups (72/1841 (3.91%) versus 81/1835 (4.41%); RR 0.90, 95% CI 0.66 to 1.23, P value = 0.50) (VISP 2004). See Analysis 1.1.

**Non-fatal or fatal stroke**

**Homocysteine-lowering interventions compared with placebo**

Meta-analysis of nine trials did not show a difference in non-fatal or fatal stroke between homocysteine-lowering interventions and placebo groups (968/22,348 (4.33%) versus 974/18,957 (5.13%); RR 0.91, 95% CI 0.82 to 1.00, P value = 0.06, $I^2$ = 11%, high quality evidence) (FOLARDA 2004; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VITATOPS 2010; WAFACS 2008; WENBIT 2008) (Analysis 1.2.). Trial sequential analysis for stroke suggests that no more trials may be needed to disprove an intervention effect of 20% relative risk reduction. Smaller risk reductions might still require further trials (Figure 7). Figure 8 shows the funnel plot for stroke.

**Figure 7. Trial sequential analysis on stroke in nine trials investigating homocysteine-lowering interventions versus placeboTrial sequential analysis of homocysteine-lowering interventions versus placebo on stroke based on the diversity-adjusted required information size (DARIS) of 17,679 patients. This DARIS was calculated based upon a proportion of patients with stroke of 5.13% in the control group; a RRR of 20% in the experimental intervention group; an alpha ($\alpha$) of 5%; a beta ($\beta$) of 20%; and a diversity of 26%. The cumulative Z-curve (blue line) temporally crosses the conventional alpha of 5%, but reverts to insignificant values. The cumulative Z-curve never crosses the trial sequential alpha-spending monitoring boundaries. After the third trial, the cumulative Z-curve crosses the trial sequential beta-spending monitoring boundary, showing that the area of futility has been reached. This suggests that no more trials may be needed to disprove an intervention effect of 20% relative risk reduction. Smaller risk reductions might still require further trials.**

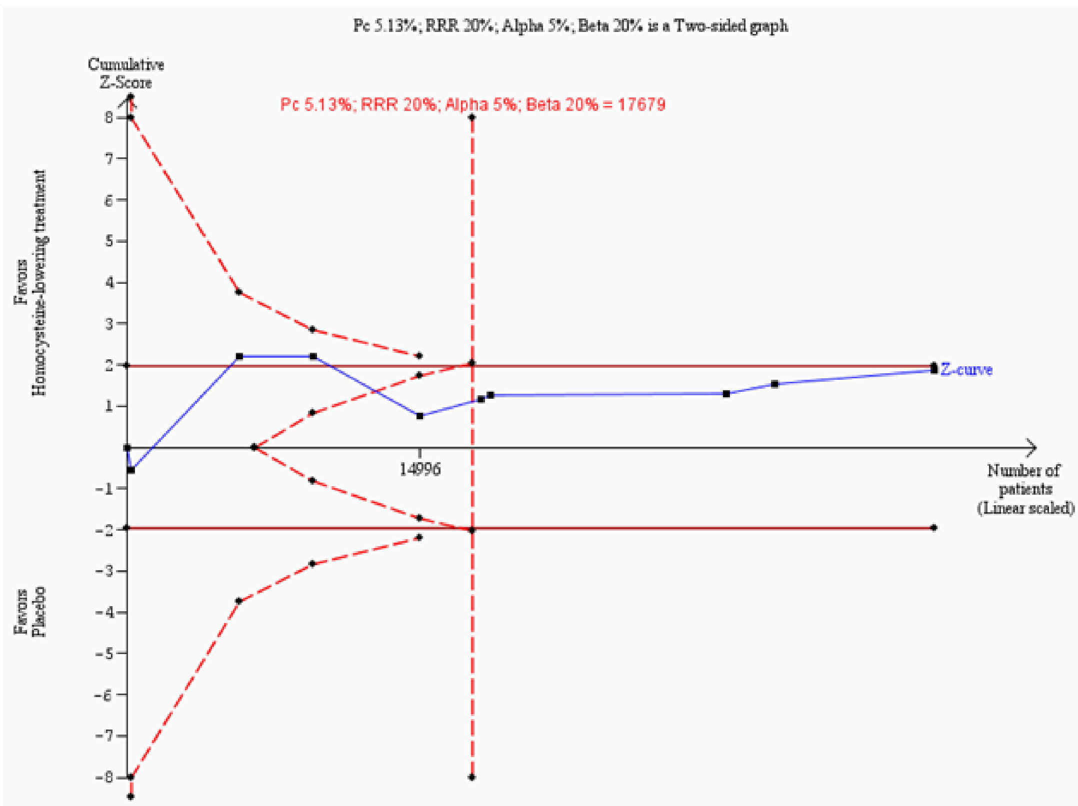
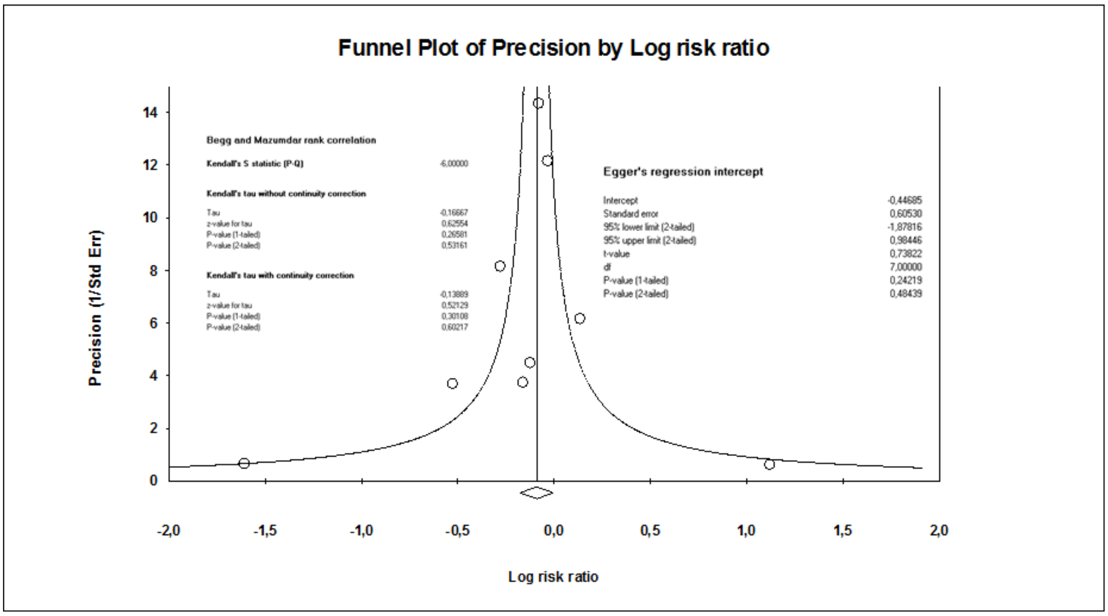


**Figure 8. Funnel plot of data from the meta-analysis of the effects of homocysteine-lowering interventions for preventing strokeThe circles show the point estimates of the included randomised clinical trials. The pattern of distribution resembles an inverted funnel. Larger trials are closer and upper to the pooled estimate. The effect sizes of the smaller trials are lower and more or less symmetrically distributed around the pooled estimate. This figure shows a low risk of publication bias.**




### Homocysteine-lowering interventions (high-dose) compared with homocysteine-lowering interventions (low-dose)

One trial found no significant difference in non-fatal or fatal myocardial infarction between intervention and control groups (152/1814 (8.37%) versus 148/1835 (8.06%); RR 1.04, 95% CI 0.84 to 1.29, P value = 0.73) (VISP 2004). See Analysis 1.2.

## Secondary outcomes

### First unstable angina pectoris episode requiring hospitalisation

Meta-analysis of four trials found no difference between intervention and placebo groups (910/8015 (11.35%) versus 468/4629 (10.11%); RR 0.98, 95% CI 0.80 to 1.21, P value = 0.87, $I^2$ = 66%) (FOLARDA 2004; HOPE-2 2006; NORVIT 2006; WENBIT 2008) (Analysis 1.3).

### Hospitalisation for heart failure

One trial found no significant difference in hospitalisation for heart failure between intervention and placebo groups (202/2758 (7.32%) versus 174/2764 (6.69%); RR 1.16, 95% CI 0.96 to 1.41, P value = 0.13) (HOPE-2 2006).

### Death from any cause

### Homocysteine-lowering interventions compared with placebo

Meta-analysis of 10 trials found no difference between intervention and placebo in mortality from any cause (2784/22,648 (12.29%) versus 2502/19,250 (12.99%); RR 1.01, 95% CI 0.96 to 1.07, P value = 0.39, $I^2$ = 6%, high quality evidence) (BVAIT 2009; FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VITATOPS 2010;

WAFACS 2008; WENBIT 2008) (Analysis 1.4). Trial sequential analysis for stroke suggests that no more trials may be needed to disprove an intervention effect of 20% relative risk reduction. Smaller risk reductions might still require further trials (Figure 9). Figure 10 shows the funnel plot for death from any cause.

**Figure 9. Trial sequential analysis on death from any cause in 10 trials investigating homocysteine-lowering interventions versus placeboTrial sequential analysis of homocysteine-lowering interventions versus placebo on death from any cause based on the diversity-adjusted required information size (DARIS) of 10,419 patients. This DARIS was calculated based upon a proportion of death from any cause out of 13% in the control group; a RRR of 15% in the experimental intervention group; an alpha ($\alpha$) of 5%; a beta ($\beta$) of 20%; and a diversity of 16%. After the third trial, the cumulative Z-curve (blue line) crosses the trial sequential beta-spending monitoring boundary, showing that the area of futility has been reached. This suggests that no more trials may be needed to disprove an intervention effect of 15% relative risk reduction. Smaller risk reductions might still require further trials.**

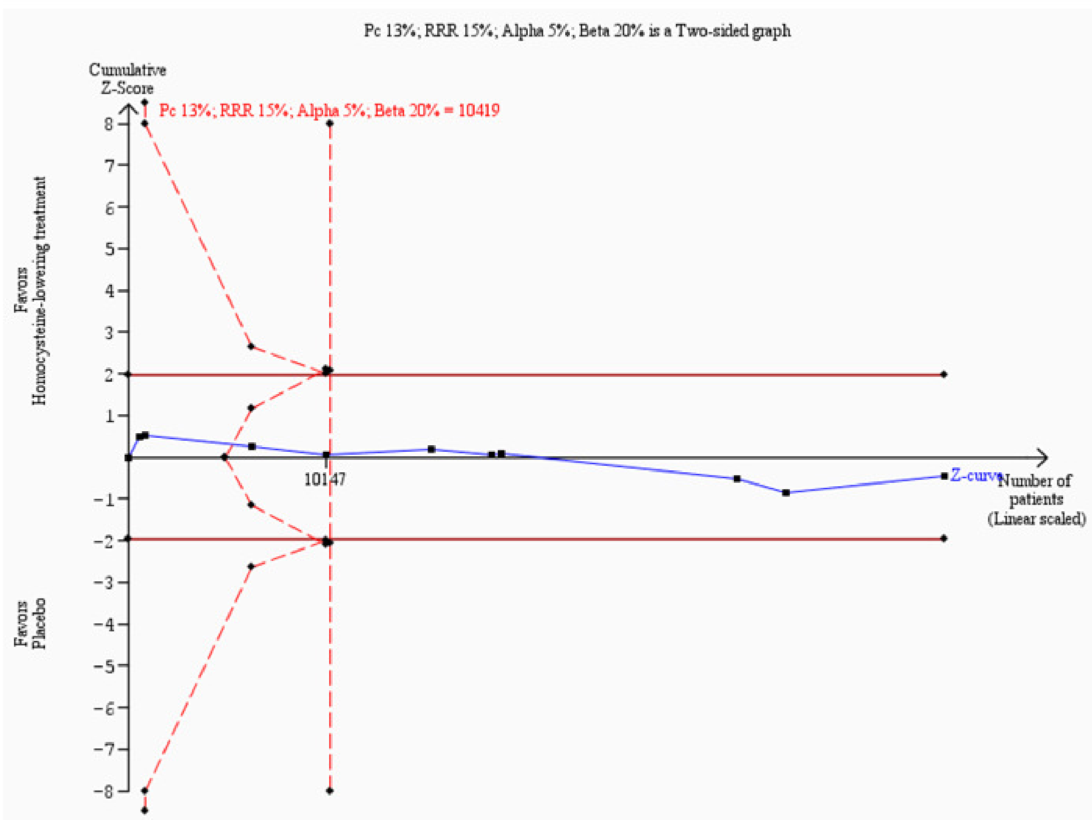
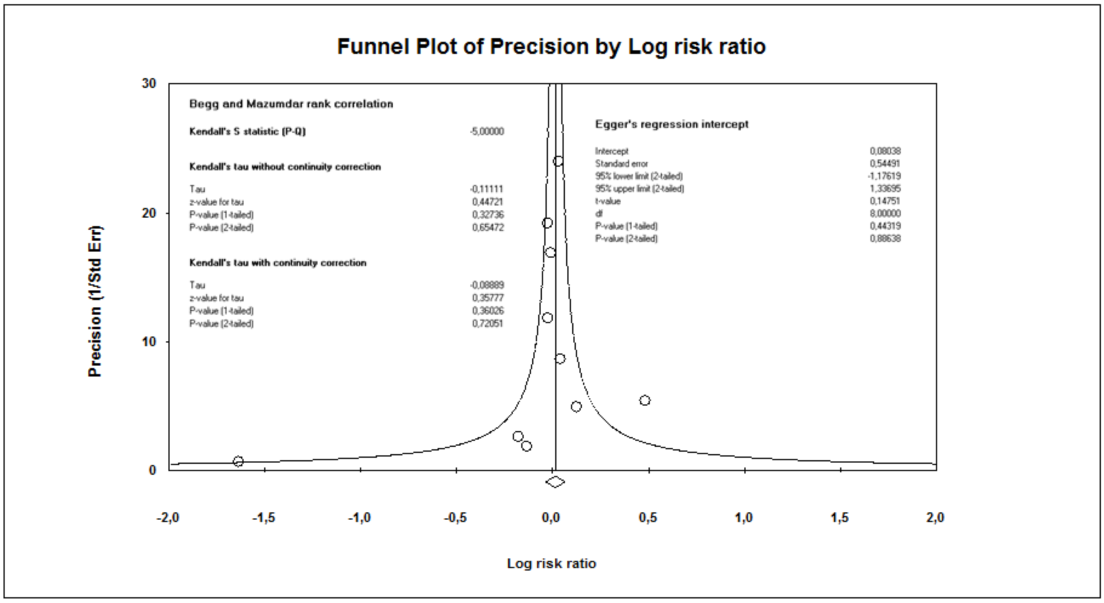


**Figure 10. Funnel plot of data from the meta-analysis of the effects of homocysteine-lowering interventions for preventing death from any causeThis figure shows a low risk of publication bias. The circles show the point estimates of the included randomised clinical trials. The pattern of distribution simulates an inverted funnel. Larger trials are closer and upper to the pooled estimate. The effect sizes of the smaller trials are lower and more or less symmetrically distributed around the pooled estimate. This figure shows a low risk of publication bias.**



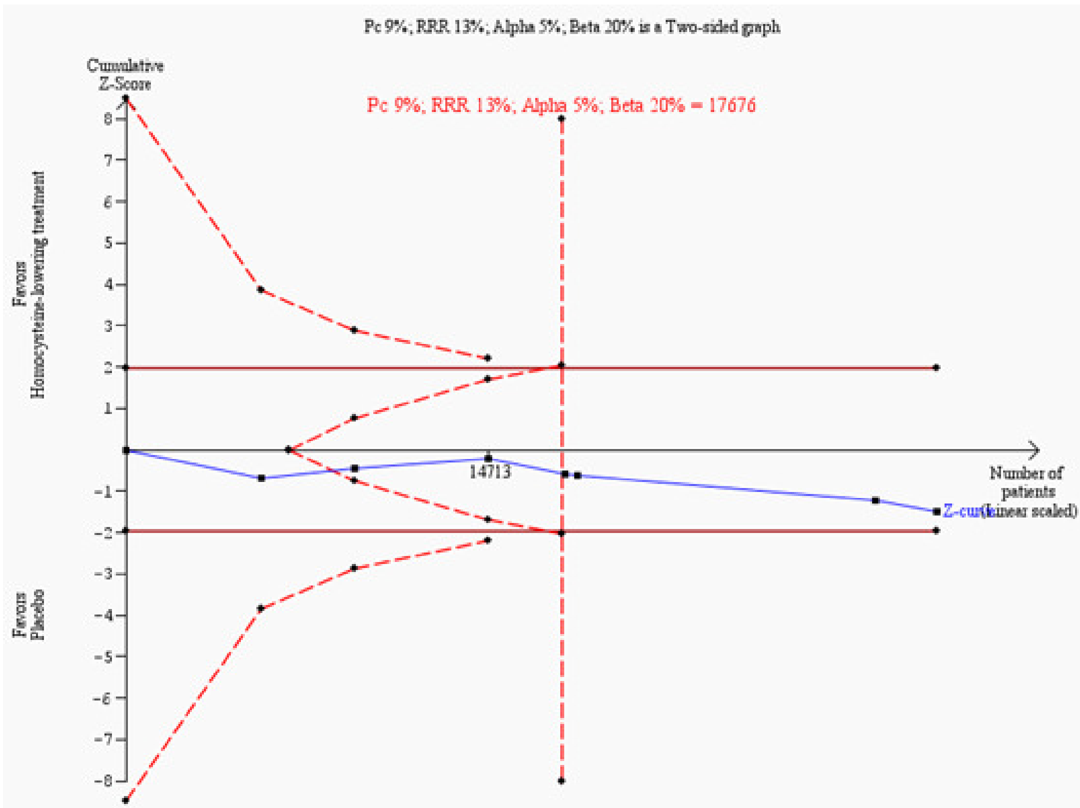

### Homocysteine-lowering interventions (high-dose) compared with homocysteine-lowering interventions (low-dose)

One trial found no significant difference in mortality from any cause between intervention and control groups (99/1814 (5.45%) versus 117/1835 (6.37%); RR 0.86, 95% CI 0.66 to 1.11, P value = 0.24) (VISP 2004). See Analysis 1.4.

#### Serious or non-serious adverse events

Meta-analysis of seven trials assessing cancer found no difference in cancer between intervention and placebo groups (1558/18,130 (8.59%) versus 1334/14,739 (9.05%); RR 1.06, 95% CI 0.98 to 1.13, P value = 0.13, $I^2$ = 0%, high quality evidence) (BVAIT 2009; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; WENBIT 2008). See Analysis 1.5. Trial sequential analysis for adverse events suggests that no more trials may be needed to disprove an intervention effect of 13% relative risk reduction (Figure 11).

**Figure 11. Trial sequential analysis on adverse events (cancer) in seven trials investigating homocysteine-lowering interventions versus placeboTrial sequential analysis of homocysteine-lowering interventions versus placebo on adverse events (cancer) based on the diversity-adjusted required information size (DARIS) of 17,676 patients. This DARIS was calculated based upon a proportion of patients developing cancer of 9% in the control group; a RRR of 13% in the experimental intervention group; an alpha ($\alpha$) of 5%; a beta ($\beta$) of 20%; and a diversity of 0%. The cumulative Z-curve (blue line) crosses the trials sequential beta-spending monitoring boundary, showing that the area of futility has been reached. This suggests that no more trials are needed to disprove an intervention effect of 13% relative risk reduction.**




## Sensitivity analysis

Heterogeneity for all main outcomes was low, as conveyed by the $I^2$ values. Moreover, very few studies were included to allow subgroup analysis. Therefore, we did not pursue extensive investigation as previously planned. Due to the lack of important heterogeneity fixed-effect and random-effects models gave the same results for the main outcomes. Sensitivity analysis included trials with low risk of bias (BVAIT 2009; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VITATOPS 2010; WAFACS 2008; WENBIT 2008).

### Primary outcomes

#### Non-fatal or fatal myocardial infarction

***Homocysteine-lowering interventions compared with placebo***

Meta-analysis of seven trials found no significant difference in non-fatal or fatal myocardial infarction between intervention and placebo groups (1707/21,960 (7.77%) versus 1219/18,572 (6.56%); RR 1.02, 95% CI 0.95 to 1.09, P value = 0.85, $I^2$ = 0%) (Analysis 2.1).

#### Non-fatal or fatal stroke

##### *Homocysteine-lowering interventions compared with placebo*

Meta-analysis of seven trials found no significant difference in non-fatal or fatal stroke between intervention and placebo groups (967/21,960 (4.40%) versus 972/18,572 (5.23%); RR 0.91, 95% CI 0.81 to 1.01, P value = 0.28, $I^2$ = 20%) (Analysis 2.2).

### Secondary outcomes

#### First unstable angina pectoris episode requiring hospitalisation

##### *Homocysteine-lowering interventions compared with placebo*

Meta-analysis of three randomised clinical trials found no significant difference in first unstable angina pectoris episode requiring hospitalisation between intervention and placebo groups (904/7875 (11.47%) versus 460/4486 (10.25%); RR 0.99, 95% CI 0.79 to 1.24, P value = 0.95, $I^2$ = 76%) (Analysis 2.3).

#### Death from any cause

##### *Homocysteine-lowering interventions compared with placebo*

Meta-analysis of eight trials found no significant difference in mortality from any cause between intervention and placebo groups (2246/22,208 (10.11%) versus 1953/18,814 (10.38%); RR 1.03, 95% CI 0.95 to 1.12, P value = 0.17, $I^2$ = 33%) (Analysis 2.4).

# DISCUSSION

## Summary of main results

This updated Cochrane Review of homocysteine-lowering interventions (B vitamins) for preventing cardiovascular events identified 12 randomised controlled trials incorporating 47,429 participants. Trials reported different combinations of homocysteine-lowering interventions compared with different control interventions. Overall, the trials had a low risk of bias and were adequately powered. Participants differed somewhat in cardiovascular risk levels (with established cardiovascular disease (CVD) or at high risk of CVD), baseline total homocysteine blood levels, access to foods fortified with folic acid or not, different dosages of vitamin and control groups, and treatment periods varying from two to seven years. We did not find significant differences on the incidence of myocardial infarction (fatal or non-fatal), stroke (fatal or non-fatal), death from any cause or adverse events (cancer).

## Overall completeness and applicability of evidence

This updated review found evidence suggesting that homocysteine-lowering interventions (vitamins B6, B12 and folic acid (B9)) are not useful for preventing cardiovascular events. We conducted a sensitivity analysis restricted to trials with low risk of bias for myocardial infarction, stroke and death from any cause. These results show consistency and are based on data from trials that included a broad range of patients with different co-morbidities who received different treatment approaches. Although these aspects could be considered as a threat to applicability, the consistency in the results derived from our analyses shows that the included trials may represent a broad picture of patients with a high risk of cardiovascular events.

This updated Cochrane Review found no new trials assessing homocysteine-lowering interventions for preventing cardiovascular events. Therefore, this update version shows the same findings as Martí-Carvajal 2013. It showed that supplementary vitamin B6, B12 and folic acid administration could not prevent cardiovascular events in patients with or without pre-existing cardiovascular disease. The trial sequential analysis for the same outcomes suggested that no more randomised trials are needed to assess the benefits and harms of homocysteine-lowering interventions for preventing cardiovascular events (Figure 5; Figure 7; Figure 9; Figure 11). Martí-Carvajal 2013 found a null effect of vitamin B-complex supplementation on cancer (Figure 11).

## Quality of the evidence

We conducted GRADE assessments on outcomes using the meta-analysed trials. Overall, the included trials had a low risk of bias (Figure 3; Figure 4).

Summary of findings for the main comparison shows the quality of evidence for homocysteine-lowering interventions compared with placebo or standard care for preventing cardiovascular events. The evidence available in this setting can be considered high quality due to the consistency of the results of the 12 trials for the main outcomes assessed (myocardial infarction, stroke and death from any cause), the precision in the pooled estimates, and the design and execution of these trials, which can be judged to be free of major threats to their validity.

## Potential biases in the review process

In a systematic review process, there are a group of biases called significance-chasing biases, such as publication bias and selective outcome reporting bias (Ioannidis 2010). Selective outcome reporting bias operates through suppression of information on specific outcomes and has similarities to study publication bias in that 'negative' results remain unpublished (Ioannidis 2010). This Cochrane Review found that overall the included randomised trials had a low risk of attrition bias and a low risk of selective outcome reporting bias (Figure 3; Figure 4).

### Agreements and disagreements with other studies or reviews

Our results are similar to the other non-Cochrane reviews (Clarke 2010; Huang 2012; Huo 2012; Ji 2013). These four reviews differ in their eligibility criteria resulting in the following: i) the inclusion by Clarke 2010, Huang 2012, Huo 2012 and Ji 2013 of the HOST trial (Jamison 2007), designed to assess the effects of homocysteine in patients with kidney or renal disease, which is out of our scope; ii) Clarke 2010 and Huo 2012 included all the trials in their pooled analysis (whereas we preferred to present the results from trials controlled with placebo separately from the results of the trials that compared different doses of homocysteine-lowering drugs (VISP 2004)); iii) it can be concluded from the Clarke 2010 publication that the authors had access to some additional data from CHAOS 2002, which we had to extract from an abstract; and finally iv) our systematic review includes five additional trials not considered in Clarke 2010, with 12,031 more patients that allowed us to obtain more accurate estimates for our outcomes of interest (BVAIT 2009; FOLARDA 2004; GOES 2003; SU.FOL.OM3 2010; VITATOPS 2010). Despite these differences, both reviews have similar results for the most relevant outcomes, which did not show significant effects derived from homocysteine-lowering drugs on cardiovascular events or overall mortality.
On the other hand, two randomised controlled trials (Jamison 2007; Vianna 2007), and one systematic review (Jardine 2012; Pan 2012) involving patients with end-stage renal disease, found no effects of homocysteine-lowering interventions for preventing cardiovascular events.
Regarding cancer, this Cochrane Review shows similar results to a recent meta-analysis involving data on 50,000 individuals (Vollset 2013). Both meta-analyses found no increased risk of cancer associated with homocysteine-lowering interventions.

## AUTHORS' CONCLUSIONS

### Implications for practice

This second update of our Cochrane Review provides evidence that homocysteine-lowering interventions do not prevent cardiovascular events. The results are based on 12 trials (47,429 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 regimens 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.

### Implications for research

The association between both the lack of clinical effectiveness and harm of homocysteine-lowering interventions might require further investigation into the other homocysteine pathways.

## ACKNOWLEDGEMENTS

We express our gratitude to the Cochrane Heart Group and peer referees for the suggestions made to enhance the quality of this review. In addition, we acknowledge Dr Temis Maria Felix for giving us permission to publish Figure 1 from Brustolin 2010, and to Carmen Verônica Abdala from BIREME/OPS/OMS for her help in developing the search strategy for LILACS. In addition, we want express our deep gratitude to Georgia Salanti for teaching us how to conduct the first version of this Cochrane Review.

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* *Indicates the major publication for the study*

# CHARACTERISTICS OF STUDIES

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

### BVAIT 2009

| | |
|---|---|
| Methods | Multicentre study: yes<br>Country: USA<br>Intention-to-treat: yes (an intention-to-treat analysis was performed for all participants who had carotid ultrasonography at baseline and at least 1 follow-up visit, page 731)<br>Unit of randomisation: patients<br>Follow-up period (years): B vitamins group (3.14 (0.48 to 4.56) versus placebo group (3.07 (0.46 to 5.0)) |
| Participants | Eligibility: 5309<br>Randomised: 506 (254 vitamins versus 252 placebo)<br>• Age (years)<br>Overall: 61.4<br>B vitamins group: 61.7 (± 10.1)<br>Placebo group: 61.1 (± 9.6)<br>• Gender (men):<br>Overall: 61%<br>B vitamins group: 61%<br>Placebo group: 61%<br>• Inclusion criteria:<br>1. Men and postmenopausal women 40 years old<br>2. Fasting tHcy 8.5 mol/L<br>3. No clinical signs/symptoms of cardiovascular disease (CVD)<br>• Exclusion criteria:<br>1. Fasting triglycerides > 5.64 mmol/L (500 mg/dL)<br>2. Diabetes mellitus or fasting serum glucose > 6.99 mmol/L (126 mg/dL)<br>3. Systolic blood pressure ≥ 160 mm Hg and/or diastolic blood pressure ≥ 100 mm Hg<br>4. Untreated thyroid disease<br>5. Creatinine clearance < 70 mL/min<br>6. Life-threatening illness with prognosis 5 years<br>7. 5 alcoholic drinks daily |
| Interventions | • HLI-intervention: folic acid (5 mg), vitamin B12 (0.4 mg) and vitamin B6 (50 mg, daily supplementation)<br>• Control: placebo<br>• Treatment duration: initial 2.5-year treatment period was extended on average 1 to 2 years |
| Outcomes | • Primary:<br>Rate of change in the right distal carotid artery intima media thickness<br>• Secondary:<br>Changes in calcium in the coronary arteries and abdominal aorta<br>• Safety:<br>1. Deaths |

| | |
|---|---|
| | 2. Cardiovascular events<br>3. Cerebrovascular events<br>4. Arterial revascularisation procedures<br>5. Cancers<br>6. Occurrence of white blood cell count below the laboratory normal limit (4000 cells/$\mu$L) |
| Notes | • Identifier: NCT00114400<br>• Conducted between 6 November 2000 and 1 June 2006<br>• A priori sample estimation: yes<br>1. Quote: ”Sample size based on carotid artery intima media thickness progression required 176 subjects/arm to detect a moderate effect size of 0.30 at 0.05 significance (2-sided) with 0.80 power. A total of 506 subjects were recruited to accommodate anticipated dropouts and initiation of lipid-lowering medications on-trial.“ (page 731)<br>• Financial disclosures: not reported<br>• Other disclosures: none<br>• Funding/support: Grant R01AG-17160 from the National Institute on Aging, National Institutes of Health. Leiner Health Products provided the B vitamin supplements and placebo<br>We sent an email to the main author of this trial in order to get the type cardiovascular event data by comparison group (4 March 2012) |

***Risk of bias***

| Bias | Authors’ judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote: ”Computer-generated random numbers were used to assign participants“ (page 731) |
| Allocation concealment (selection bias) | Low risk | Quote: ”Computer-generated random numbers were used to assign participants“ (page 731) |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote: ”Participants, clinical staff, imaging specialists, and data monitors were masked to treatment assignment.“ (page 731) |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Quote: ”...imaging specialists, ... were masked to treatment assignment.“ (page 731). & ”Scans were analyzed without knowledge of treatment assignment using validated calcium scoring software“ (for secondary outcome)” (page 731)<br>Comments: the main outcomes were to assess the impact of the HLI on reduction of subclinical atherosclerosis progression |

BVAIT 2009 (*Continued)*

| | | |
|---|---|---|
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | • B vitamins group<br>1. Lost to follow-up (n = 27): brain tumour (n = 1), medical problems (n = 2), refused methionine test (n = 1), active military duty (n = 1), too busy (n = 22)<br>2. Discontinued intervention (n = 8): attributed intervention to a medical problem (n = 1), medical problem (n = 2), wanted to take vitamins (n = 1), too busy (n = 4)<br>• Placebo group<br>1. Lost to follow-up (n = 27): died (n = 2), medical problems (n = 4), refused methionine test (n = 1), active military duty (n = 1), too busy (n = 19)<br>2. Discontinued Intervention (n = 7): attributed intervention to a medical problem (n = 1), medical problem (n = 3), wanted to take vitamins (n = 2), too busy (n = 1)<br>• Evaluable included in analysis:<br>1. B vitamins group: 97.6% (248/254)<br>2. Placebo group: 96% (242/252)<br>• Completed the initially planned (2.5-year trial period): 8.1% (446/506): (88% (223/446) B vitamin; 88% (223/446) placebo) |
| Selective reporting (reporting bias) | Low risk | The study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified. We also checked www.clinicaltrials.gov and the ID number was: NCT00114400 |
| Other bias | Low risk | - |

**CHAOS 2002**

| | |
|---|---|
| Methods | Multicentre study<br>Follow-up period: mean of 1.7 years |
| Participants | 1882 patients randomised (folic acid: 942 versus placebo: 940 patients)<br>• Gender: not reported<br>• Age: not reported<br>• Homocysteine levels at baseline (treatment group) ($\mu$mol/L): 11.2 ± 6.9 $\mu$mol/L<br>• Inclusion criteria (1 of the following):<br>1. Positive coronary angiogram |

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|---|---|
| | 2. Admission with MI or unstable angina<br>• Exclusion criteria: not reported |
| Interventions | • Intervention: folic acid 5 mg per day<br>• Control: placebo in addition to usual drugs<br>Treatment duration: 2 years |
| Outcomes | Composite outcome: MI, revascularisation, death from cardiovascular cause |
| Notes | • Sponsors: not available<br>• Other: data not yet fully published. Results in the table correspond to conference proceedings<br>Homocysteine levels were only collected in 2 participating centres |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Described as randomised<br>Insufficient information about the sequence generation process to permit judgement of 'Low risk' or 'High risk' Data not yet fully published. Results in the table correspond to conference proceedings |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information about the sequence generation process to permit judgement of 'Low risk' or 'High risk' Data not yet fully published. Results in the table correspond to conference proceedings |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Unclear risk | Described as double-blinded. However, the information was obtained from the final report (abstract)<br>Insufficient information to permit judgement of 'Low risk' or 'High risk' |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Described as double-blinded. However, the information was obtained from the final report (abstract)<br>Insufficient information to permit judgement of 'Low risk' or 'High risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | Unclear risk | Flow of participants during trial was not reported. Data not yet fully published. Results in the table correspond to conference proceedings |

**CHAOS 2002** (*Continued)*

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|---|---|---|
| Selective reporting (reporting bias) | Low risk | The study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified |
| Other bias | Unclear risk | Insufficient information to assess whether an important risk of bias exists |

**FOLARDA 2004**

| | |
|---|---|
| Methods | Multicentre study<br>Country: The Netherlands<br>Follow-up period: 1 year |
| Participants | 283 randomised patients (folic acid: 140 versus standard care: 143)<br>• Gender (% men): folic acid: 69% versus standard care: 70%<br>• Age (mean): folic acid: 59 years versus standard care: 59<br>• Homocysteine levels at baseline: not reported<br>• Inclusion criteria (1 of the following):<br>1. Myocardial infarction<br>2. Total cholesterol value at admission or within 24 hours after onset of symptoms: > 6.5 $\mu$mol/L (251 mg/dL)<br>3. Elevation of CK-MB at least 2 times upper the limit of normal function<br>4. Markedly increased chest pain lasting more than 30 minutes or classical ECG changes<br>• Exclusion criteria:<br>1. Age under 18 years,<br>2. Use of lipid-lowering agents within the previous 3 months<br>3. High triglyceride levels > 4.5 $\mu$mol/L<br>4. Known familial dyslipidaemia<br>5. Low vitamin B12 levels<br>6. Hyperhomocysteinaemia (total plasma homocysteine > 18 $\mu$mol/L) or a known disturbed methionine loading test (total plasma homocysteine > 47 $\mu$mol/L)<br>7. Severe renal failure (serum creatinine > 180 $\mu$mol/L)<br>8. Hepatic disease<br>9. Severe heart failure (New York Heart Association class IV)<br>10. Scheduled percutaneous coronary intervention or coronary artery bypass graft operation |
| Interventions | • Intervention:<br>Folic acid: 5 mg per day<br>Treatment was initiated at least 1 day prior to hospital discharge, and no later of 14 days after the MI. The treatment continued for 1 year. Patients in this group also received statin therapy (fluvastatin, 40 mg per day). The clinician had at their discretion the prescription of additional prophylactic medication (aspirin, beta-blocking agents and/or ACE inhibitors)<br>• Control:<br>Standard care: statin therapy (fluvastatin, 40 mg per day). The clinician had at their |

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| | discretion the prescription of additional prophylactic medication (aspirin, beta-blocking agents and/or ACE inhibitors)<br>• Treatment duration: 1 year |
| Outcomes | 1. Cardiovascular death (sudden death, fatal recurrent MI, fatal stroke and other cardiovascular deaths)<br>2. Non-cardiovascular death<br>3. Recurrent MI<br>4. Recurrent ischaemia requiring hospitalisation or revascularisation |
| Notes | Study phase: III<br>• A priori sample estimation: sample size calculation to detect (80% power and 5% significance level) a 50% reduction in clinical events in that kind of patients, assuming a 1-year event rate of 30%. These numbers resulted in an estimation of 120 patients per group. Analyses conducted on ITT basis<br>• Sponsors: AstraZeneca, The Netherlands, Working Group on Cardiovascular research, The Netherlands. One author is an Established Investigator of the Netherlands Heart Foundation<br>• Other: author did not perform homocysteine-level measures during the study |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Quote: "patients were randomised..."<br>Insufficient information about the sequence generation process to permit judgement of 'Low risk' or 'High risk' |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information to permit judgement of 'Low risk' or 'High risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | High risk | Quote: "... treatment with open label folic acid [...] or not" |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Quote: "An Independent Data and Safety Monitoring Committee adjudicated all major clinical events." |
| Incomplete outcome data (attrition bias)<br>All outcomes | High risk | 23 patients discontinued treatment and no information is given |
| Selective reporting (reporting bias) | Low risk | The study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified |

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| Other bias | Unclear risk | Insufficient information to permit judgement of ’Low risk’ or ’High risk’ |

**GOES 2003**

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|---|---|
| Methods | Single-centre study<br>Country: The Netherlands<br>Follow-up period: 1 year |
| Participants | 593 randomised patients (folic acid: 300 versus standard care: 293)<br>• Gender (% men): folic acid: 76% versus standard care: 80%<br>• Age (mean ± SD): folic acid: 64.9 ± 9.9 versus standard care: 65.5 ± 9.7<br>• Homocysteine levels at baseline: not reported<br>• Inclusion criteria:<br>1. Myocardial infarction<br>2. Coronary artery lesions (> 60%) on coronary angiography<br>3. Percutaneous coronary intervention<br>4. Coronary artery bypass graft surgery<br>5. Patients had to be stable, with no invasive vascular procedures scheduled<br>6. Statin therapy for at least 3 months<br>7. Taking any form of vitamin B-containing medication, regularly or sporadically<br>• Exclusion criteria:<br>1. Age < 18 years<br>2. History of low vitamin B12 levels<br>3. Therapy for hyperhomocysteinaemia<br>4. Severe renal failure, or any other treatment for renal disease<br>5. Hepatic disease<br>6. Severe heart failure (New York Heart Association functional class IV)<br>7. Serious illness that would exclude follow-up time of at least 3 years |
| Interventions | • Intervention: folic acid: 0.5 mg per day<br>• Control group: standard care<br>• 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<br>• Treatment duration: not reported |
| Outcomes | • **Primary (composite)**:<br>1. Composite: vascular death (sudden death, fatal recurrent MI, fatal stroke and other cardiovascular deaths)<br>2. Non-cardiovascular death<br>3. Recurrent acute coronary syndromes<br>4. Invasive coronary procedures<br>5. Cerebrovascular accident or transient ischaemic attack<br>6. Any other vascular surgery (carotid endarterectomy, abdominal aneurysmectomy, or peripheral vascular surgery including limb amputation for vascular reasons)<br>• **Secondary**:<br>1. Hospitalisation for unstable angina |

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| Notes | • Study phase: III<br>• A priori sample size estimation: (80% power and 5% significance level) to detect a 50% reduction in clinical events in that type of patients, assuming a 2-year event rate of 15%. These numbers resulted in an estimation of 300 patients per group. Analyses conducted on ITT basis<br>• Sponsors: trial with public funding (Stichting Paracard)<br>• 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***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote: "A computer program randomly allocated patients [...] to treatment" |
| Allocation concealment (selection bias) | Unclear risk | No information reported about this domain |
| Blinding of participants and personnel (performance bias)<br>All outcomes | High risk | Quote: "... treatment with open label folic acid [...] or standard care." |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Quote: "Adjudication of all clinical events was performed by an independent end point monitoring committee unaware of treatment arm." |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | After randomisation, 12 patients per group withdrew from the study but were followed up and included in the final analysis |
| Selective reporting (reporting bias) | Low risk | The study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified |
| Other bias | Unclear risk | Insufficient information to permit judgement of 'Low risk' or 'High risk' |

**HOPE-2 2006**

| | |
|---|---|
| Methods | Multicentre international study (13 countries; 145 centres)<br>Follow-up period: 5 years |
| Participants | 5522 patients randomised (vitamin: 2758 versus placebo group: 2764 patients)<br>• Gender (% men): vitamin: 71.1% versus placebo: 72.4%<br>• Age (mean ± SD): vitamin: 68.8 ± 7.1 versus placebo: 68.9 ± 6.8 |

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| | • Homocysteine level at baseline: 12.2 $\mu$mol/L (1.6 mg/L)<br>• Inclusion criteria:<br>1. Men and women aged > 55 years<br>2. History 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)<br>• Exclusion criteria:<br>1. Patients taking vitamin supplements containing more than 0.2 mg of folic acid per day |
| Interventions | Intervention:<br>• Multivitamin therapy with 2.5 mg of folic acid, 50 mg of vitamin B6 and 1 mg of vitamin B12 per day<br>Control:<br>• Matching placebo daily<br>Treatment duration: 5 years |
| Outcomes | Primary outcome (composite):<br>• Death from cardiovascular causes, myocardial infarction, stroke<br>• Secondary outcomes:<br>• Total ischaemic events (composite of death from cardiovascular causes, myocardial infarction, stroke, hospitalisation for unstable angina and revascularisation)<br>• Death from any cause<br>• Hospitalisation for unstable angina or congestive heart failure<br>• Revascularisation<br>• Incidence and death for cancer<br>• Other outcomes: transient ischaemic attacks, venous thromboembolic events, fractures |
| Notes | • Study phase: III, registered (ClinicalTrials.gov number NCT00106886)<br>• Sample calculation a priori: yes. Sample size calculation to detect between a 17% and a 20% reduction (80% and 90% power, respectively) in the risk rate of the 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 on ITT basis<br>• 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***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote: "The study used central telephone randomization" |

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|---|---|---|
| Allocation concealment (selection bias) | Low risk | Centralised telephone randomisation (accessible 24 hours a day) |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote: "All study investigators, personnel, and participants were unaware of the randomization procedure and the treatment assignments."<br>Vitamins manufactured to be indistinguishable in colour, weight or ability to be dissolved in water |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | This trial assessed objective outcomes |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | 21 patients in the treatment group and 16 in the placebo group did not complete the study<br>Vital status known for 99.3% of the sample |
| Selective reporting (reporting bias) | Low risk | The study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified |
| Other bias | Low risk | |

**NORVIT 2006**

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|---|---|
| Methods | Multicentre study<br>Country: Norway<br>Follow-up period: 3.5 years |
| Participants | 3749 patients randomised (folic acid, vitamins B6 and B12: 937 versus folic acid, vitamin B12: 935 versus vitamin B6: 934 versus placebo: 943)<br>• Gender (% men):<br>Folic acid, vitamins B6 and B12: 73%<br>Folic acid, vitamin B12: 74%<br>Vitamin B6: 73%<br>Placebo: 75%<br>• Age (mean ± SD, years)<br>Folic acid, vitamins B6 and B12: 63.6 ± 11.9<br>Folic acid, vitamin B12: 63.2 ± 11.6<br>Vitamin B6: 62.5 ± 11.7<br>Placebo: 62.6 ± 11.4 years<br>• Inclusion criteria:<br>1. Men and women aged 30 to 85 years,<br>2. History of acute MI within 7 days before randomisation |

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| | • Exclusion criteria:<br>1. Coexisting disease associated with a life expectancy < 4 years<br>2. Prescribed treatment with B vitamins or untreated vitamin B deficiency<br>3. Inability to follow the protocol, as judged by the investigator |
| Interventions | • Intervention:<br>1. Folic acid (group 1): 0.8 mg; vitamin B12: 0.4 mg; vitamin B6: 40 mg per day<br>2. Folic acid (group 2): 0.8 mg; vitamin B12: 0.4 mg per day<br>3. Vitamin B6 (group 3): 40 mg per day<br>• Control: placebo<br>Medication was delivered in single capsules taken once per day. For the first 2 weeks after study entry patients in groups 1 and 2 received an additional folic acid dose (5 mg) per day, whereas the other 2 groups received placebo<br>Treatment duration: not clearly described |
| Outcomes | • Primary outcome (composite):<br>1. Recurrent MI, stroke and sudden death attributed to coronary artery disease<br>• Secondary outcomes:<br>1. Myocardial infarction<br>2. Unstable angina pectoris requiring hospitalisation<br>3. Coronary revascularisation with percutaneous coronary intervention or coronary artery bypass grafting<br>4. Stroke<br>5. Death from any cause<br>Incident cases of cancer |
| Notes | • Study phase: III, registered (ClinicalTrials.gov number NCT00266487)<br>• A priori sample size estimation: yes. Sample size calculation to detect a 20% relative reduction in the rate of primary endpoint (assuming 25% of endpoints in the placebo group). These numbers resulted in an estimation of 3500 patients assuming 750 primary events<br>The calculation of the sample size was based on data from previous Scandinavian trials, assuming the 3-year rate of the primary endpoint would be 25% 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 statistical power of more than 90% to detect a 20% relative reduction in the rate of the primary endpoint, given a 2-sided alpha value of 0.05<br>• 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***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|

NORVIT 2006 (*Continued*)

| | | |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | No information reported about this domain |
| Allocation concealment (selection bias) | Low risk | The manufacturer provided centrally study sites with blocks of medication assigned in numerical order |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | All study personnel and participants were unaware of the treatment assignments<br>Vitamins were manufactured to be indistinguishable in colour, weight or ability to be dissolved in water |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Quote: "All end points were adjudicated by members of the end-points committee, who were unaware of patients' treatment assignments." |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | 11% of patients stopped the medication<br>94% attended the final visit, but data on mortality were available for the entire sample. Incomplete outcome data for 20 patients<br>Patients that had not completed the planned follow-up were followed up by phone or consulted for vital status |
| Selective reporting (reporting bias) | Low risk | The study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified |
| Other bias | Low risk | - |

**SEARCH 2010**

| | |
|---|---|
| Methods | Multicentre study (88 sites)<br>Country: United Kingdom<br>Intention-to-treat: yes<br>Unit of randomisation: patients were survivors of MI<br>Follow-up period: 6.7 ± 1.5 person-years |
| Participants | Clinical condition: survivors of myocardial infarction in secondary care hospitals<br>1. Potential participants invited by mail: 83,237<br>2. Attended screening visit: 34,780<br>3. Entered pre-randomisation run-in-phase: 19,190. Quote: "Run-in treatment involved placebo vitamin tablets (and 20 mg simvastatin daily, which allowed baseline lipid levels to be assessed after all participants had received the same statin therapy) |

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| | (page 2487)<br>4. Randomised: 12,064 (folic acid and B12: 6033 versus placebo: 6031)<br>• Gender (% men)<br>Men: 10,012<br>Women: 2052<br>1. Folic acid and B12: 83%<br>2. Placebo: 83%<br>• Age (at randomisation)<br>Mean (SD) age of 64.2 (8.9) years<br>Folic acid and vitamin B12:<br>1. < 60 years: 31%<br>2. ≥ 60 years to < 70 years: 40%<br>3. ≥ 70 years: 29%<br>Placebo:<br>1. < 60 years: 31%<br>2. ≥ 60 years to < 70 years: 40%<br>3. ≥ 70 years: 29%<br>• Inclusion criteria:<br>1. Men and women<br>2. Aged 18 to 80 years<br>3. History of myocardial infarction<br>4. Had no clear indication for folic acid<br>5. Blood cholesterol levels of at least 135 mg/dL if already taking a statin medication or 174 mg/dL if not (to convert cholesterol to mmol/L, multiply by 0.0259)<br>• Exclusion criteria:<br>1. Chronic liver, renal or muscle disease<br>2. History of any cancer (except non-melanoma skin cancer)<br>3. Use of potentially interacting medications |
| Interventions | • Intervention: 1 tablet daily containing 2 mg folic acid plus 1 mg vitamin B12<br>• Control: placebo<br>Both medications were supplied in specially prepared calendar packs (and, separately, using a 2 x 2 factorial design, either 80 mg or 20 mg simvastatin daily) |
| Outcomes | • Primary outcome (composite):<br>1. Incidence of first major vascular event, defined as non-fatal MI or death from coronary heart disease, fatal or non-fatal stroke, or any arterial revascularisation<br>• Secondary outcomes:<br>1. Major vascular events in the first year after randomisation (when little difference was anticipated) and, separately, in the later years of the treatment period<br>2. Major vascular events among participants subdivided into 3 similar-sized groups with respect to blood homocysteine levels at the end of the pre-randomisation run-in period (before any study vitamin treatment had been taken)<br>3. Major vascular events in the presence of one or other of the allocated study simvastatin regimens<br>4. Major coronary events, defined as non-fatal MI, death from coronary disease, or coronary revascularisation<br>5. Any type of stroke (excluding transient ischaemic attacks)<br>• Tertiary outcomes: |

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| | 1. Total and cause-specific mortality (considering vascular and non-vascular causes separately)<br>2. Vascular mortality excluding the first year after randomisation<br>3. Coronary and non-coronary revascularisation separately<br>4. Confirmed haemorrhagic and other strokes separately<br>5. Pulmonary embolus<br>6. Total and site-specific cancers<br>7. Hospitalisations for various other causes<br>8. Adverse effects of treatment |
| Notes | • Identifier: ISRCTN 74348595<br>• Reason for a pre-randomisation run-in phase: to limit subsequent randomisation to those likely to take the randomly allocated study treatment for several years (page 2487)<br>• Conducted between September 1998 and June 2008<br>• A priori sample estimation: yes<br>1. Quote: "It was prespecified in the protocol that the steering committee could modify the study plans while still blinded to the event rates in each treatment group." (page 2488)<br>2. Quote: "in 2004, blind to interim results for clinical outcomes, the steering committee decided to change the primary outcome from major coronary events to major vascular events and to continue until at least 2800 patients had had a confirmed major vascular event in order to have 90% power at P.05 to detect a 10% reduction in risk." (page 2489)<br>3. Comment: assumptions for sample size estimation were based on Boushey 1995; Bowman 2007; HSC 2002 and SSSS 1994<br>• Financial disclosures: reported<br>• Funding/support: Quote: "The study was funded by Merck (manufacturers of simvastatin and suppliers of the vitamins). The CTSU also receives core support from the UK Medical Research Council and the British Heart Foundation." (page 2493)<br>• Role of sponsors: Quote: "The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, and approval of the manuscript. The University of Oxford acted as the sponsor of the study." (page 2493)<br>• Additional information: http://www.searchinfo.org/SEARCH_protocol.pdf |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote: "The central telephone randomization system used a minimization algorithm to balance the treatment groups with respect to major prognostic factors." (page 2487) |
| Allocation concealment (selection bias) | Low risk | Quote: "The central telephone randomization system used a minimization algorithm to balance the treatment groups with re- |

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|---|---|---|
| | | spect to major prognostic factors." (page 2487) |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote: " All such information was reviewed by coordinating center clinicians who were unaware of the study treatment allocation and events coded according to prespecified criteria" (page 2487) |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | No blinding of outcome assessment, but the review authors judge that the outcome measurement is not likely to be influenced by lack of blinding |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | Vitamin group: 98.9% (5970/6033) completed follow-up<br>Placebo group: 99.1% (5975/6031) completed follow-up |
| Selective reporting (reporting bias) | Low risk | The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way |
| Other bias | Low risk | - |

**SU.FOL.OM3 2010**

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|---|---|
| Methods | Multicentre study (257 sites)<br>Country: France<br>Intention-to-treat: yes. "All analyses were conducted according to the principle of intention to treat" (page 2)<br>Unit of randomisation: patients with a history of ischaemic heart disease or stroke<br>Follow-up period: median: 4.7 years; mean 4.2 ± 1.0 years |
| Participants | Clinical condition: patients with a history of ischaemic heart disease or stroke<br>1. Patients assessed for eligibility: 3374<br>2. Randomised: 2501 (B vitamins plus omega 3 fatty acids: 620, omega 3 fatty acids: 633, B vitamins: 622, and placebo: 626)<br>3. Complete follow-up: 2222 (89%)<br>• Gender (% men)<br>Men: 1987<br>Women: 514<br>1. B vitamins plus omega 3 fatty acids: 79.5%<br>2. Omega 3 fatty acids: 79.2%<br>3. B vitamins: 79.9%<br>4. Placebo: 79.2% |

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| | • Age<br>Mean (SD) age of 60.9 (8.8) years.<br>1. B vitamins plus omega 3 fatty acids: 60.5 (53.9 to 68.9)<br>2. Omega 3 fatty acids: 60.41 (5.7 to 68.7)<br>3. B vitamins: 60.7 (54.7 to 68.3)<br>4. Placebo: 60.9 (54.5 to 68.1)<br>• Inclusion criteria:<br>1. Men and women<br>2. Aged 45 to 80 years<br>3. History of acute coronary or cerebral ischaemic event within the 12 months before randomisation<br>• Exclusion criteria:<br>1. Age (< 45 years or > 80 years)<br>2. Ill-defined diagnosis of cardiovascular disease<br>3. Inability or unwillingness to comply with study treatment<br>4. Disease or treatment that might interfere with metabolism of homocysteine or omega 3 fatty acids, in particular methotrexate for treating cancer or rheumatoid arthritis and chronic renal failure (plasma creatinine concentration > 200 mol/L or creatinine clearance < 40 mL/min)<br>5. Individuals with transient ischaemic attacks |
| Interventions | • Intervention: 1 tablet daily containing 5-methyltetrahydrofolate (560 $\mu$g), vitamin B6 (3 mg) and B12 (20 $\mu$g)<br>• Control: placebo<br>Furthermore: supplement containing omega 3 polyunsaturated fatty acids (600 mg of eicosapentaenoic acid and docosahexaenoic acid at a ratio of 2:1) |
| Outcomes | • Primary outcome (composite):<br>1. First major cardiovascular event: non-fatal myocardial infarction, ischaemic stroke or death from cardiovascular disease (including fatal myocardial infarction, stroke, sudden death (within 1 hour of onset of acute symptoms in the absence of violence or accident), aortic dissection, cardiac failure or other fatal event defined by the medical committee as having a cardiovascular cause)<br>• Secondary outcomes:<br>1. Acute coronary syndrome without myocardial infarction<br>2. Resuscitation from sudden death<br>3. Coronary artery bypass surgery<br>4. Coronary angioplasty<br>5. Cardiac failure<br>6. Ventricular arrhythmia<br>7. Supraventricular arrhythmia<br>8. Cardiac surgery of any kind, transient ischaemic attack<br>9. Deep vein thrombosis<br>10. Pulmonary embolism<br>11. Carotid surgery or carotid artery angioplasty<br>12. Peripheral arterial surgery or angioplasty<br>13. Any vascular procedure<br>14. Death from all causes |

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|---|---|
| Notes | • Identifier: ISRCTN 41926726<br>• Conducted between 1 February 2003 and 1 June 2007<br>• A priori sample estimation: yes<br>1. Quote: "The sample size was calculated for the estimated eventrate of 0.087 in the placebo group, based on the event rates observed in previous trials in similar populations and in epidemiological studies. No interaction between B vitamins and omega 3 fatty acids was anticipated. The planned enrolment of 2500 participants with an average follow-up of five years was expected to have more than 90% power to detect a 10% reduction in the relative risk of major vascular events associated with B vitamins or omega 3 fatty acids and a 19% reduction for the combination of omega 3 fatty acids and B vitamins, given a two sided $\alpha$ value of 0.05." (page 3)<br>Comment: assumptions were based on Galan et al (HSC 2002; SU.FOL.OM3 2010; Yusuf 2000)<br>• Competing interest: reported<br>• Funding/support: Quote: "The SU.FOL.OM3 trial was supported by the French Ministry of Research (R02010JJ), Ministry of Health (DGS), Sodexo, Candia, Unilever, Danone, Roche Laboratory, Merck EPROVA GS, and Pierre Fabre Laboratory." (page 8)<br>• The supplements were provided without charge by Merck Eprova AG (5-methyltetrahydrofolate), Roche Laboratory (vitamins B6 and B12), and Pierre Fabre (omega 3 fatty acids). The gelatin capsules were manufactured by Catalent Pharma Solutions (Beinheim, France) (page 2) |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote: "Randomisation was performed by means of a computerised block sequence stratified by three age groups (44 - 54, 55 - 64, and 65 - 80 years), sex, prior disease at enrolment (myocardial infarction, acute coronary syndrome, or ischaemic stroke) and recruitment centre. Permuted block randomisation (with block size randomly selected as 8) was used." (page 2) |
| Allocation concealment (selection bias) | Low risk | Quote: "Randomisation was performed by means of a computerised block sequence stratified by three age groups (44 - 54, 55 - 64, and 65 - 80 years), sex, prior disease at enrolment (myocardial infarction, acute coronary syndrome, or ischaemic stroke) and recruitment centre. Permuted block randomisation (with block size randomly selected as 8) was used." (page 2) |

| | | |
|---|---|---|
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote: "Patients, clinicians, trial coordinators, and outcome investigators were blinded to treatment allocation." (page 2)<br>Quote: "treatment capsules for one year (and repeated yearly) in an appropriately labelled package." (page 2) |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Quote: "... and outcome investigators were blinded to treatment allocation." (page 2)<br>Quote: "All events were adjudicated by two independent committees of cardiologists or neurologists who were blinded to treatment allocation." (page 3) |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | 1. B vitamins plus omega 3 fatty acids: 11.8% (547/620)<br>2. Omega 3 fatty acids: 9.6% (572/633)<br>3. B vitamins: 12.6% (542/622)<br>4. Placebo: 10.4% (561/626)<br>Comments: reasons for losses were reported |
| Selective reporting (reporting bias) | Low risk | The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have reported in the pre-specified way. "This study is registered with Current Controlled Trials (No ISRCTN41926726" (page 3) |
| Other bias | Low risk | - |

**VISP 2004**

| | |
|---|---|
| Methods | Country: USA, Canada and Scotland<br>Multicentre international study<br>Follow-up period: 2 years |
| Participants | 3680 randomised (high-dose: 1827 versus low-dose: 1853)<br>Gender (% men): high-dose: 62.3% versus low-dose: 62.8%<br><br>Age (mean ± SD): high-dose: 66.4 (10.8) versus low-dose: 66.2 (10.8)<br>• Inclusion criteria:<br>1. Non-disabling ischaemic stroke (Modified Rankin Stroke Scale 3): onset 120 days before randomisation. Focal neurological deficit of likely atherothrombotic origin, classified as ischaemic stroke by questionnaire/algorithm or confirmed as new cerebral infarction consistent with symptoms by cranial computed tomography or brain magnetic resonance imaging |

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|---|---|
| | 2. Total homocysteine level 25th percentile for North American stroke population<br>3. Age: ≥ 35 years<br>4. Accessibility for follow-up<br>5. Agreement to take study medication and not take other multivitamins or pills containing folic acid or vitamin B6<br>6. Written informed consent<br>• Exclusion criteria:<br>1. Potential sources of emboli (atrial fibrillation within 30 days of stroke, prosthetic cardiac valve, intracardiac thrombus or neoplasm, or valvular vegetation)<br>2. Other major neurological illness that would obscure evaluation of recurrent stroke<br>3. Life expectancy 2 years<br>4. Renal failure requiring dialysis<br>5. Untreated anaemia or untreated vitamin B12 deficiency<br>6. Systolic blood pressure 185 mm Hg or diastolic blood pressure 105 mm Hg on 2 readings 5 minutes apart at time of eligibility determination<br>7. Refractory depression, severe cognitive impairment, or alcoholism or other substance abuse<br>8. 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<br>9. Childbearing potential<br>10. Participation in another trial with active intervention<br>11. General anaesthesia 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 randomisation or scheduled to be performed within 30 days after randomisation |
| Interventions | • High-dose multivitamin therapy<br>2.5 mg folic acid; 0.4 mg vitamin B12; 25 mg vitamin B6 per day<br>• Low-dose multivitamin therapy<br>20 micrograms folic acid; 6 micrograms vitamin B12; 200 micrograms vitamin B6 per day<br>• Co-interventions:<br>1. Risk factor control education<br>2. Aspirin (325 mg/d)<br>Duration of treatment: not described |
| Outcomes | • Primary outcome:<br>1. Recurrent cerebral infarction<br>• Secondary outcomes:<br>1. Coronary heart disease, including: myocardial infarction requiring hospitalisation; coronary revascularisation; and fatal coronary heart disease<br>2. Death |
| Notes | • Study phase: III<br>• A priori sample size estimation: yes. Sample size calculation (80% power at 0.05 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 8% of events in the first year and 4% in the second year, with 20% losses to follow-up). These numbers resulted in an estimation of 1800 patients per group. Trialists planned up to 6 interim analyses |

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| | • 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 |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | The allocation of participants was programmed by the statistical co-ordinating centre, encrypted and entered into a data entry program installed on a study computer at each site |
| Allocation concealment (selection bias) | Low risk | Allocation programmed by the statistical co-ordinating centre. All the information on assignment were encrypted an entered in computers in study sites<br>After verification of eligibility participants were assigned in 1 of 20 medication codes |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | The drug distributor centre bottled and distributed the vitamins, which were manufactured to be indistinguishable in colour, weight or ability to be dissolved in water |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | The primary endpoint was reviewed by a local neurologist and 2 external independent review neurologists |
| Incomplete outcome data (attrition bias)<br>All outcomes | Unclear risk | 132 patients in the low-dose group and 133 in the high-dose group 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 group discontinued the assigned treatment<br>Patients who had not completed the planned follow-up were invited to an exit visit |
| Selective reporting (reporting bias) | Low risk | The study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified |

| | | |
|---|---|---|
| Other bias | Low risk | - |

**VITATOPS 2010**

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|---|---|
| Methods | Multicentre study: 123 medical centres (20 countries) from 4 continents<br>Follow-up period (median and interquartile range, years): 3.4 (2.0 to 5.5)<br>Intention-to-treat: yes<br>Unit of randomisation: patients with recent stroke or transient ischaemic attack within the past 7 months |
| Participants | 8164 randomised<br>4089 received folic acid and vitamins B (B6 and B12)<br>4075 received placebo<br>• Age (mean ± SD; years)<br>1. Overall: 62.6 ± 12.5<br>2. Vitamin: 62.5 ± 12.6<br>3. Placebo: 62.6 ± 12.4<br>• Gender (men):<br>1. Overall: 64%<br>2. Vitamin: 64% (2614/4089)<br>3. Placebo: 64% (2604/4075)<br>• Inclusion criteria:<br>1. Stroke (ischaemic or haemorrhagic) or transient ischaemic attack (eye or brain), as defined by standard criteria, within the past 7 months<br>2. Patients with haemorrhagic stroke<br>• Exclusion criteria:<br>1. Taking folic acid, vitamin B6, vitamin B12 or a folate antagonist (e.g. methotrexate)<br>2. Pregnant or women of childbearing potential<br>3. Patients with limited life expectancy (e.g. because of ill health) |
| Interventions | • Intervention:<br>1. Folic acid: 2 mg/d<br>2. Vitamin $B_6$: 25 mg/d<br>3. Vitamin $B_{12}$: 0.5 mg/d<br>• Control: placebo<br>Co-interventions: not reported |
| Outcomes | • Primary outcome (composite): whichever occurred first<br>1. Non-fatal stroke<br>2. Non-fatal myocardial infarction<br>3. Death from any vascular causes<br>• Secondary outcomes:<br>1. Stroke (non-fatal or fatal)<br>2. Myocardial infarction (non-fatal or fatal)<br>3. Death from any vascular cause<br>4. Death from any cause<br>5. Revascularisation procedures |

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|---|---|
| | 6. The composite of non-fatal stroke, non-fatal myocardial infarction and death from any vascular cause<br>7. Revascularisation procedures of the coronary, cerebral or peripheral circulation |
| Notes | • Identifier numbers: NCT00097669 and ISRCTN74743444<br>• Date of study: 19 November 1998 to 31 December 2008<br>• A priori sample size estimation: yes. Quote: "equally sized intervention and placebo groups, a minimum follow-up of 6 months for the last patient to be randomly allocated, an annual primary outcome event rate of 8% in the placebo group, and a 15% decrease in the relative risk of the primary outcome among patients assigned to B vitamins (i.e., 6.8% per year) compared with placebo. For a type 1 error of 5% and type 2 error of 20%, and assuming a mean follow-up of 2 years, a sample size of 3982 patients was required in each treatment group." (page 857). Comment: assumption for estimating annual primary outcome event rate in the placebo groups was based on CAPRIE 1996<br>• Sponsor: Australia National Health and Medical Research Council, UK Medical Research Council, Singapore Biomedical Research Council, Singapore National Medical Research Council, Australia National Heart Foundation, Royal Perth Hospital Medical Research Foundation and Health Department of Western Australia<br>• Rol of Sponsor: "The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, the writing of the report, or in the decision to submit the paper for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication." (page 858)<br>• Conflicts of interest: reported<br>• Vitamin tablets and matching placebo tablets were supplied by Blackmores, Australia (page 864)<br>• All investigator-reported outcomes and adverse events were audited by a masked adjudication committee (page 857) |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote: "Random allocation was done by use of a central 24 hrs telephone service or an interactive website by use of random permuted blocks stratified by hospital" (page 856) |
| Allocation concealment (selection bias) | Low risk | Quote: "Random allocation was done by use of a central 24 hrs telephone service or an interactive website by use of random permuted blocks stratified by hospital" (page 856) |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote: "Patients, clinicians, trial coordinators, and outcome investigators were masked to treatment allocation" (page 856) |

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| | | Quote: ”had the same colour and coating“ (page 856) |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Quote: ”...and outcome investigators were masked to treatment allocation“ (page 856) |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | Loss to final follow-up:<br>Global: 8.6% (702/8164)<br>B vitamins group: 8.5% (348/4089)<br>Placebo group: 8.7% (354/4075)<br>Comment: reasons for losses were reported |
| Selective reporting (reporting bias) | Low risk | The study protocol is not available but it is clear that published reports include all expect outcomes, including those that were pre-specified. This trial is registered with ClinicalTrials.gov, NCT00097669 and Current Controlled Trials, ISRCTN74743444.” (page 858) |
| Other bias | Low risk | - |

**WAFACS 2008**

| | |
|---|---|
| Methods | Multicentre study<br>Country: USA<br>Follow-up period: 7.3 years |
| Participants | N: 5442 randomised patients (vitamin group: 2721 patients; placebo group: 2721 patients)<br>• Gender: women health professionals<br>• Age (mean (SD)) years<br>Active group: 62.8 (8.8)<br>Control group: 62.8 (8.8)<br>• Inclusion criteria<br>1. Women<br>2. Age: 40 years or older<br>3. Postmenopausal or had no intention of becoming pregnant<br>4. History of CVD or had at least 3 cardiac risk factors<br>• Exclusion criteria:<br>1. Cancer (excluding non-melanoma skin cancer) within the past 10 years<br>2. Serious non-cardiovascular disease<br>3. Warfarin or other anticoagulants use |
| Interventions | • Intervention:<br>Folic acid: 2.5 mg; vitamin B12: 1 mg; vitamin B6: 50 mg per day<br>• Control:<br>Matching placebo per day |

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| | • Co-interventions: vitamin C, vitamin E, ß-carotene<br>• Treatment duration: not clearly reported |
| Outcomes | • Primary (composite):<br>1. Incident myocardial infarction, stroke, coronary revascularisation procedures (coronary artery bypass grafting or percutaneous coronary intervention) and cardiovascular mortality<br>• Secondary:<br>1. Myocardial infarction rate<br>2. Stroke rate<br>3. Total coronary heart disease events (myocardial infarction, coronary revascularisation and death from coronary heart disease) |
| Notes | • Study phase: III, registered (ClinicalTrials.gov number NCT00000541)<br>• The information in this table was kindly supplied by Dr. Nancy Cook who was the statistician for the WACS and WAFACS studies (23 June 2008)<br>The WACS study was a 2 x 2 x 2 factorial trial of 3 antioxidants, vitamins C, E and beta-carotene. Randomisation 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<br>• A priori sample size estimation: sample size with 91.5% power to detect a 20% reduction in the primary endpoint (major vascular events). For the endpoints of total CHD (defined as non-fatal MI, CHD death or revascularisation), MI and stroke, the minimum detectable risk reduction with 80% power ranges from 19% to 32%. A 2-sided significance level of 0.05 was used<br>• 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)<br>• 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<br>• Other: the analyses of the endpoints were done only for these confirmed outcomes. However, there were an additional 43 recorded deaths for total mortality |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Block randomisation with a block size of 8 generated by computer, stratified by age |

| | | |
|---|---|---|
| Allocation concealment (selection bias) | Low risk | Central randomisation. Patients were sent yearly supplies of calendar packs containing their medication or matching placebos identical in appearance |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | All study investigators, personnel and participants were unaware of the participants' treatment assignments<br>Patients were sent packs containing medication or matching placebos identical in appearance<br>An independent committee monitored the "safety and overall quality and scientific integrity" of the trial, which was blinded to treatment assignment<br>All the information was supplied by Nancy Cook (WACS statistician, 23 June 2008) |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | An independent committee monitored the "safety and overall quality and scientific integrity" of the trial, which was blinded to treatment assignment<br>All the information was supplied by Nancy Cook (WACS statistician, 23 June 2008)<br>Comments: this trial had objective outcomes |
| Incomplete outcome data (attrition bias)<br>All outcomes | Unclear risk | Unknown vital status for 194 patients in the folic acid group and 207 patients in the placebo group. All the patients were included in the primary analysis, but how was not described |
| Selective reporting (reporting bias) | Low risk | The study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified |
| Other bias | Low risk | - |

**WENBIT 2008**

| | |
|---|---|
| Methods | Multicentre study<br>Country: Norway<br>Follow-up period: 4 years |
| Participants | 3096 patients randomised (folic acid, vitamins B6 and B12: 772 versus folic acid, vitamin B12: 772 versus vitamin B6: 772 versus placebo: 780) |

| | |
|---|---|
| | • Gender (% men)<br>1. Folic acid, vitamins B6 and B12: 81.2%<br>2. Folic acid, vitamin B12: 80.4%<br>3. Vitamin B6: 80.2%<br>4. Placebo: 76.5%<br>• Age (mean ± SD, years):<br>1. Folic acid, vitamins B6 and B12: 61.7 ± 10.3<br>2. Folic acid, vitamin B12: 61.3 ± 10.0<br>3. Vitamin B6: 61.4 ± 9.7<br>4. Placebo: 62.0 ± 9.9<br>• Inclusion criteria:<br>1. Age: 18 years or older<br>2. Undergoing coronary angiography for suspected coronary artery disease and/or aortic valve stenosis at the 2 university hospitals in western Norway<br>• Exclusion criteria:<br>1. Unavailability for follow-up<br>2. Participation in other trials<br>3. History of alcohol abuse, serious mental illness or cancer |
| Interventions | • Intervention:<br>1. Folic acid (group 1): 0.8 mg; vitamin B12: 0.4 mg; vitamin B6: 40 mg per day<br>2. Folic acid (group 2): 0.8 mg; vitamin B12: 0.4 mg per day<br>3. Vitamin B6 (group 3): 40 mg per day<br>• Control: placebo<br>• Co-interventions: statins, insulin, aspirin, clopidogrel, beta-blockers, ACE inhibitors/ARBs, calcium channel blockers, loop diuretics, oral antidiabetics, medication for chronic obstructive pulmonary disease<br>Duration of treatment: not described |
| Outcomes | • Primary outcome (composite):<br>1. All-cause death, non-fatal acute myocardial infarction, acute hospitalisation for unstable angina pectoris and non-fatal thromboembolic stroke<br>• Secondary outcomes:<br>1. Acute myocardial infarction<br>2. Acute hospitalisation for angina pectoris<br>3. Stable angina pectoris with angiographically verified progression<br>4. Myocardial revascularisation procedures<br>5. Stroke<br>6. Incident cases of cancer |
| Notes | • Study phase: III, registered (ClinicalTrials.gov number NCT00354081)<br>• A priori sample size estimation: sample of 3088 participants to detect a 20% reduction in the primary endpoint during 4 years of follow-up with a statistical power of 80% at a 2-sided significance level of 0.05<br>• 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 |

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| | 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<br>• 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<br>• 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<br>• This trial was stopped due to no beneficial effects and a suggested increased risk of cancer from B vitamin treatment |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | 2 x 2 factorial design with block randomisation, with a block size of 20 |
| Allocation concealment (selection bias) | Low risk | Centralised independently by the manufacturer (Alpharma)<br>Study nurses received coded boxes provided to participants in numerical order. The codes were kept by the manufacturer until eligibility data were complete |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Vitamins were manufactured to be indistinguishable in colour, weight or ability to be dissolved in water. Endpoints adjudicated by an independent committee unaware of patient's assignment |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Quote: "end-points committees were unaware of the treatment allocation" |
| Incomplete outcome data (attrition bias)<br>All outcomes | High risk | 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 86% of patients at the final visit |
| Selective reporting (reporting bias) | Low risk | The study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified |

**WENBIT 2008** (*Continued)*

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|---|---|---|
| Other bias | Low risk | - |

ACE: angiotensin-converting enzyme
ARB: angiotensin receptor blockers
CAD: coronary artery disease
CHD: coronary heart disease
CK-MB: creatine kinase-MB
CVD: cardiovascular disease
ECG: electrocardiogram
HLI: homocysteine-lowering interventions
ITT: intention-to-treat
MI: myocardial infarction
RCT: randomised controlled trial
SD: standard deviation
t-Hcy: total homocysteine

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

| Study | Reason for exclusion |
|---|---|
| Bazzano 2006 | Systematic review |
| Clarke 2010 | Systematic review |
| Cui 2010 | Observational study |
| Deshmukh 2010 | Randomised clinical trial that did not assess patient-oriented outcomes and excluded the pre-defined outcomes for this Cochrane Review |
| Durga 2011 | Randomised clinical trial that did not assess patient-oriented outcomes and excluded the pre-defined outcomes for this Cochrane Review |
| Earnest 2012 | Randomised clinical trial with follow-up of less than 1 year |
| Ebbing 2009 | Combined analyses of NORVIT 2006 and WENBIT 2008 |
| Ebbing 2009a | Combined analyses of NORVIT 2006 and WENBIT 2008 |
| FINEST 2006 | Randomised clinical trial with follow-up of less than 1 year |
| Green 2010 | Randomised clinical trial that did not assess patient-oriented outcomes and excluded the pre-defined outcomes for this Cochrane Review |
| Holmes 2011 | Meta-analysis of genetic studies and randomised trials |

(*Continued*)

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|---|---|
| Huang 2012 | Systematic review |
| Huo 2012 | Systematic review |
| Imasa 2009 | Randomised clinical trial with follow-up of less than 1 year |
| Jardine 2012 | Systematic review in people with kidney disease |
| Ji 2013 | Systematic review of randomised clinical trials |
| Lange 2004 | Randomised clinical trial with follow-up of less than 1 year |
| Lee 2010 | Systematic review |
| Lonn 2007 | Narrative review |
| Mager 2009 | Observational study |
| Manolescu 2010 | Narrative review |
| Mei 2010 | Systematic review of randomised clinical trials including pre-existing cardio-cerebrovascular or renal disease patients |
| Miller 2010 | Systematic review |
| Moghaddasi 2010 | Case-control study |
| Méndez-González 2010 | Narrative review |
| Ntaios 2009 | Narrative review |
| Ntaios 2010 | Randomised clinical trial that did not assess patient-oriented outcomes such as was pre-defined for this Cochrane Review |
| PACIFIC 2002 | Randomised clinical trial with follow-up of less than 1 year |
| Pan 2012 | Systematic review |
| Rautiainen 2010 | Observational study |
| Sharifi 2010 | Randomised clinical trial that did not assess patient-oriented outcomes and excluded the pre-defined outcomes for this Cochrane Review |
| Shidfar 2009 | Randomised clinical trial that evaluated the effects of folate supplementation on lowering homocysteine levels and changes in total antioxidant capacity in asymptomatic hypercholesteraemic adults under lovastatin treatment. It did not include the pre-defined outcomes for this Cochrane Review |
| Sudchada 2012 | Systematic review |

(*Continued*)

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|---|---|
| Swiss 2002 | Randomised clinical trial with follow-up of less than 1 year |
| Tighe 2011 | Randomised clinical trial that evaluated the effects of folate supplementation on lowering homocysteine levels. It did not include the pre-defined outcomes for this Cochrane Review |
| Vesin 2007 | Narrative review |
| Wang 2007 | Systematic review |
| Wang 2012 | Systematic review |
| Wierzbicki 2007 | Narrative review |
| Yang 2012 | Systematic review |
| Zappacosta 2013 | Randomised clinical trial that did not assess patient-oriented outcomes and excluded the pre-defined outcomes for this Cochrane Review |
| Zhang 2009 | Systematic review |
| Zhang 2013 | Systematic review |
| Zhou 2011 | Systematic review |

# 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 | 12 | | Risk Ratio (M-H, Random, 95% CI) | Subtotals only |
| 1.1 Homocysteine-lowering versus placebo | 11 | 43780 | Risk Ratio (M-H, Random, 95% CI) | 1.02 [0.95, 1.10] |
| 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 | 10 | | Risk Ratio (M-H, Random, 95% CI) | Subtotals only |
| 2.1 Homocysteine-lowering treatment versus placebo | 9 | 41305 | Risk Ratio (M-H, Random, 95% CI) | 0.91 [0.82, 1.00] |
| 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 hospitalisation | 4 | 12644 | Risk Ratio (M-H, Random, 95% CI) | 0.98 [0.80, 1.21] |
| 4 Death from any cause | 11 | | Risk Ratio (M-H, Random, 95% CI) | Subtotals only |
| 4.1 Homocysteine-lowering treatment versus placebo | 10 | 41898 | Risk Ratio (M-H, Random, 95% CI) | 1.01 [0.96, 1.07] |
| 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) | 7 | 32869 | Risk Ratio (M-H, Random, 95% CI) | 1.06 [0.98, 1.13] |

**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 | 7 | 40532 | Risk Ratio (M-H, Random, 95% CI) | 1.02 [0.95, 1.09] |
| 1.1 Trials with low risk of bias (mixed populations) | 6 | 35090 | Risk Ratio (M-H, Random, 95% CI) | 1.02 [0.95, 1.10] |
| 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 | 7 | 40532 | Risk Ratio (M-H, Random, 95% CI) | 0.91 [0.81, 1.01] |
| 2.1 Trials with low risk of bias (mixed populations) | 6 | 35090 | Risk Ratio (M-H, Random, 95% CI) | 0.89 [0.81, 0.98] |
| 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] |

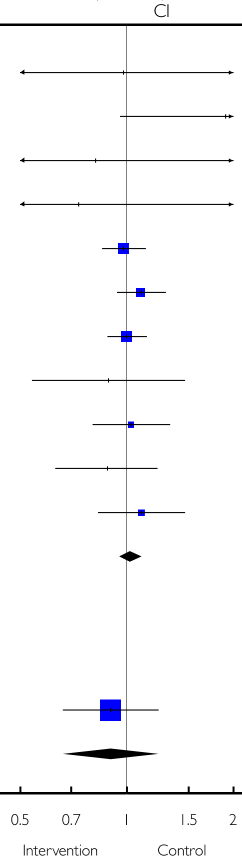
| | | | | |
|---|---|---|---|---|
| 3 First unstable angina pectoris episode requiring hospitalisation | 3 | 12361 | Risk Ratio (M-H, Random, 95% CI) | 0.99 [0.79, 1.24] |
| 4 Death from any cause | 8 | 41022 | Risk Ratio (M-H, Random, 95% CI) | 1.03 [0.95, 1.12] |
| 4.1 Trials with low risk of bias (mixed populations) | 7 | 35580 | Risk Ratio (M-H, Random, 95% CI) | 1.05 [0.95, 1.15] |
| 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




| Study or subgroup | Intervention n/N | Control n/N | Risk Ratio M-H,Random,95% CI | Weight | Risk Ratio M-H,Random,95% CI |
|---|---|---|---|---|---|
| 1 Homocysteine-lowering versus placebo | | | | | |
| BVAIT 2009 | 2/248 | 2/242 | | 0.1 % | 0.98 [ 0.14, 6.87 ] |
| CHAOS 2002 | 23/942 | 12/940 | | 1.1 % | 1.91 [ 0.96, 3.82 ] |
| FOLARDA 2004 | 8/140 | 10/143 | | 0.6 % | 0.82 [ 0.33, 2.01 ] |
| GOES 2003 | 3/300 | 4/293 | | 0.2 % | 0.73 [ 0.17, 3.24 ] |
| HOPE-2 2006 | 341/2758 | 349/2764 | | 26.1 % | 0.98 [ 0.85, 1.13 ] |
| NORVIT 2006 | 534/2806 | 163/943 | | 20.1 % | 1.10 [ 0.94, 1.29 ] |
| SEARCH 2010 | 431/6033 | 429/6031 | | 30.7 % | 1.00 [ 0.88, 1.14 ] |
| SU.FOL.OM3 2010 | 28/1242 | 32/1259 | | 2.0 % | 0.89 [ 0.54, 1.46 ] |
| VITATOPS 2010 | 118/4089 | 114/4075 | | 7.9 % | 1.03 [ 0.80, 1.33 ] |
| WAFACS 2008 | 65/2721 | 74/2721 | | 4.7 % | 0.88 [ 0.63, 1.22 ] |
| WENBIT 2008 | 190/2311 | 58/779 | | 6.4 % | 1.10 [ 0.83, 1.46 ] |
| **Subtotal (95% CI)** | **23590** | **20190** | | **100.0 %** | **1.02 [ 0.95, 1.10 ]** |
| Total events: 1743 (Intervention), 1247 (Control) | | | | | |
| Heterogeneity: Tau$^2$ = 0.0; Chi$^2$ = 6.27, df = 10 (P = 0.79); I$^2$ =0.0% | | | | | |
| Test for overall effect: Z = 0.56 (P = 0.58) | | | | | |
| 2 Homocysteine-lowering treatment at high dose versus low dose | | | | | |
| VISP 2004 | 72/1814 | 81/1835 | | 100.0 % | 0.90 [ 0.66, 1.23 ] |
| **Subtotal (95% CI)** | **1814** | **1835** | | **100.0 %** | **0.90 [ 0.66, 1.23 ]** |

(*Continued . . .*)

(... Continued)

| Study or subgroup | Intervention n/N | Control n/N | Risk Ratio M-H,Random,95% CI | Weight | Risk Ratio M-H,Random,95% CI |
|---|---|---|---|---|---|
| Total events: 72 (Intervention), 81 (Control) | | | | | |
| Heterogeneity: not applicable | | | | | |
| Test for overall effect: Z = 0.67 (P = 0.50) | | | | | |
| Test for subgroup differences: $Chi^2$ = 0.61, df = 1 (P = 0.44), $I^2$ =0.0% | | | | | |

0.5 0.7 1 1.5 2

Intervention Control

## 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 treatment versus other (any comparisons)

Outcome: 2 Stroke

| Study or subgroup | Intervention 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 | | | | | |
| BVAIT 2009 | 0/248 | 2/242 | | 0.1 % | 0.20 [ 0.01, 4.04 ] |
| FOLARDA 2004 | 1/140 | 0/143 | | 0.1 % | 3.06 [ 0.13, 74.58 ] |
| HOPE-2 2006 | 111/2758 | 147/2764 | | 15.0 % | 0.76 [ 0.59, 0.96 ] |
| NORVIT 2006 | 71/2806 | 27/943 | | 5.1 % | 0.88 [ 0.57, 1.37 ] |
| SEARCH 2010 | 277/6033 | 286/6031 | | 28.1 % | 0.97 [ 0.82, 1.14 ] |
| SU.FOL.OM3 2010 | 21/1242 | 36/1259 | | 3.5 % | 0.59 [ 0.35, 1.01 ] |
| VITATOPS 2010 | 360/4089 | 388/4075 | | 35.2 % | 0.92 [ 0.81, 1.06 ] |
| WAFACS 2008 | 79/2721 | 69/2721 | | 9.2 % | 1.14 [ 0.83, 1.57 ] |
| WENBIT 2008 | 48/2311 | 19/779 | | 3.6 % | 0.85 [ 0.50, 1.44 ] |
| **Subtotal (95% CI)** | **22348** | **18957** | | **100.0 %** | **0.91 [ 0.82, 1.00 ]** |
| Total events: 968 (Intervention), 974 (Control) | | | | | |
| Heterogeneity: $Tau^2$ = 0.00; $Chi^2$ = 9.01, df = 8 (P = 0.34); $I^2$ =11% | | | | | |

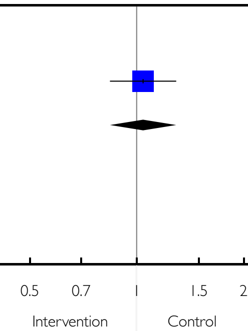
0.5 0.7 1 1.5 2

Intervention Control

(Continued ...)

(. . . Continued)

| Study or subgroup | Intervention n/N | Control n/N | Risk Ratio M-H,Random,95% CI | Weight | Risk Ratio M-H,Random,95% CI |
|---|---|---|---|---|---|
| Test for overall effect: Z = 1.88 (P = 0.060) | | | | | |
| 2 Homocysteine-lowering treatment at high dose versus low dose | | | | | |
| VISP 2004 | 152/1814 | 148/1835 | | 100.0 % | 1.04 [ 0.84, 1.29 ] |
| **Subtotal (95% CI)** | **1814** | **1835** | | **100.0 %** | **1.04 [ 0.84, 1.29 ]** |
| Total events: 152 (Intervention), 148 (Control) | | | | | |
| Heterogeneity: not applicable | | | | | |
| Test for overall effect: Z = 0.35 (P = 0.73) | | | | | |



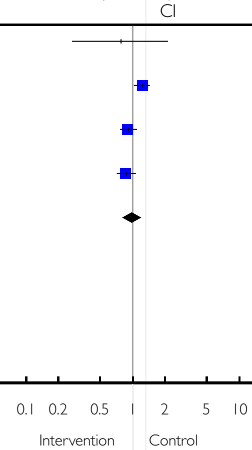

## Analysis 1.3. Comparison 1 Homocysteine-lowering treatment versus other (any comparisons), Outcome 3 First unstable angina pectoris episode requiring hospitalisation.

Review: Homocysteine-lowering interventions for preventing cardiovascular events

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

Outcome: 3 First unstable angina pectoris episode requiring hospitalisation

| Study or subgroup | Intervention 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 (Intervention), 468 (Control) | | | | | |
| Heterogeneity: $Tau^2 = 0.03$; $Chi^2 = 8.72$, df = 3 (P = 0.03); $I^2 = 66\%$ | | | | | |
| Test for overall effect: Z = 0.16 (P = 0.87) | | | | | |
| Test for subgroup differences: Not applicable | | | | | |



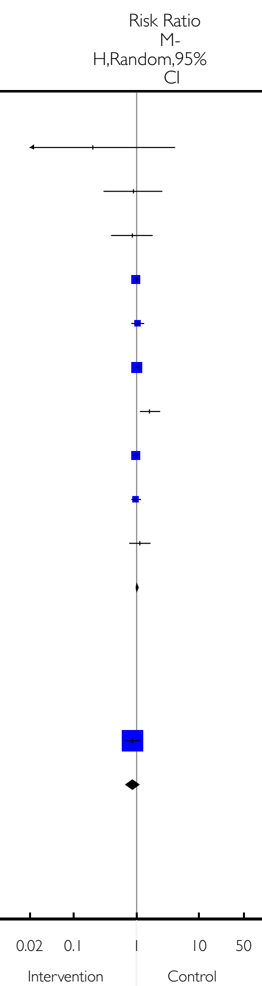
## Analysis 1.4. Comparison 1 Homocysteine-lowering treatment versus other (any comparisons), Outcome 4 Death from any cause.

Review: Homocysteine-lowering interventions for preventing cardiovascular events

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

Outcome: 4 Death from any cause

| Study or subgroup | Intervention n/N | Control n/N | Weight | Risk Ratio M-H,Random,95% CI |
|---|---|---|---|---|
| 1 Homocysteine-lowering treatment versus placebo | | | | |
| BVAIT 2009 | 0/248 | 2/242 | 0.0 % | 0.20 [ 0.01, 4.04 ] |
| FOLARDA 2004 | 6/140 | 7/143 | 0.3 % | 0.88 [ 0.30, 2.54 ] |
| GOES 2003 | 12/300 | 14/293 | 0.5 % | 0.84 [ 0.39, 1.78 ] |
| HOPE-2 2006 | 470/2758 | 475/2764 | 19.6 % | 0.99 [ 0.88, 1.11 ] |
| NORVIT 2006 | 276/2806 | 89/943 | 5.7 % | 1.04 [ 0.83, 1.31 ] |
| SEARCH 2010 | 983/6033 | 951/6031 | 35.0 % | 1.03 [ 0.95, 1.12 ] |
| SU.FOL.OM3 2010 | 72/1242 | 45/1259 | 2.3 % | 1.62 [ 1.13, 2.33 ] |
| VITATOPS 2010 | 614/4089 | 633/4075 | 24.4 % | 0.97 [ 0.87, 1.07 ] |
| WAFACS 2008 | 250/2721 | 256/2721 | 10.3 % | 0.98 [ 0.83, 1.15 ] |
| WENBIT 2008 | 101/2311 | 30/779 | 1.9 % | 1.13 [ 0.76, 1.69 ] |
| **Subtotal (95% CI)** | **22648** | **19250** | **100.0 %** | **1.01 [ 0.96, 1.07 ]** |
| Total events: 2784 (Intervention), 2502 (Control) | | | | |
| Heterogeneity: Tau$^2$ = 0.00; Chi$^2$ = 9.59, df = 9 (P = 0.38); I$^2$ =6% | | | | |
| Test for overall effect: Z = 0.46 (P = 0.65) | | | | |
| 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 ]** |
| Total events: 99 (Intervention), 117 (Control) | | | | |
| Heterogeneity: not applicable | | | | |
| Test for overall effect: Z = 1.17 (P = 0.24) | | | | |



## 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)

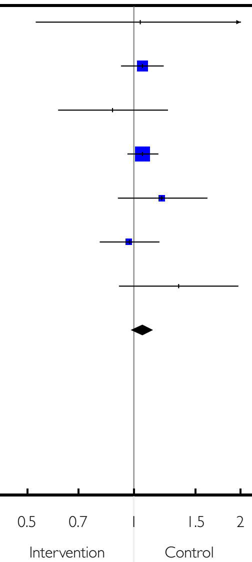
| Study or subgroup | Intervention n/N | Control n/N | Risk Ratio M-H,Random,95% CI | Weight | Risk Ratio M-H,Random,95% CI |
|---|---|---|---|---|---|
| BVAIT 2009 | 16/248 | 15/242 | | 1.1 % | 1.04 [ 0.53, 2.06 ] |
| HOPE-2 2006 | 358/2758 | 340/2764 | | 25.6 % | 1.06 [ 0.92, 1.21 ] |
| NORVIT 2006 | 104/2806 | 40/943 | | 3.9 % | 0.87 [ 0.61, 1.25 ] |
| SEARCH 2010 | 678/6033 | 639/6031 | | 47.3 % | 1.06 [ 0.96, 1.17 ] |
| SU.FOL.OM3 2010 | 92/1253 | 77/1259 | | 5.8 % | 1.20 [ 0.90, 1.61 ] |
| WAFACS 2008 | 187/2721 | 192/2721 | | 13.1 % | 0.97 [ 0.80, 1.18 ] |
| WENBIT 2008 | 123/2311 | 31/779 | | 3.3 % | 1.34 [ 0.91, 1.97 ] |
| **Total (95% CI)** | **18130** | **14739** | | **100.0 %** | **1.06 [ 0.98, 1.13 ]** |

Total events: 1558 (Intervention), 1334 (Control)

Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 3.94$, df = 6 (P = 0.68); $I^2 = 0.0\%$

Test for overall effect: Z = 1.50 (P = 0.13)

Test for subgroup differences: Not applicable



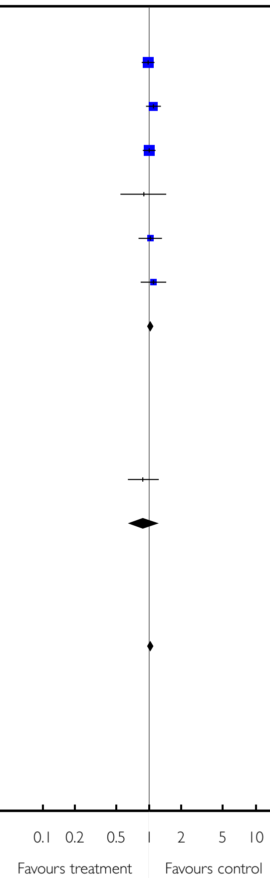
## Analysis 2.1. Comparison 2 Homocysteine-lowering treatment versus other (Sensitivity analysis), Outcome 1 Myocardial infarction.

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 | | 26.7 % | 0.98 [ 0.85, 1.13 ] |
| NORVIT 2006 | 534/2806 | 163/943 | | 20.5 % | 1.10 [ 0.94, 1.29 ] |
| SEARCH 2010 | 431/6033 | 429/6031 | | 31.3 % | 1.00 [ 0.88, 1.14 ] |
| SU.FOL.OM3 2010 | 28/1242 | 32/1259 | | 2.1 % | 0.89 [ 0.54, 1.46 ] |
| VITATOPS 2010 | 118/4089 | 114/4075 | | 8.1 % | 1.03 [ 0.80, 1.33 ] |
| WENBIT 2008 | 190/2311 | 58/779 | | 6.5 % | 1.10 [ 0.83, 1.46 ] |
| **Subtotal (95% CI)** | **19239** | **15851** | | **95.2 %** | **1.02 [ 0.95, 1.10 ]** |
| Total events: 1642 (Treatment), 1145 (Control) | | | | | |
| Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 1.87$, df = 5 (P = 0.87); $I^2$ =0.0% | | | | | |
| Test for overall effect: Z = 0.61 (P = 0.54) | | | | | |
| 2 Trials with low risk of bias (only women included) | | | | | |
| WAFACS 2008 | 65/2721 | 74/2721 | | 4.8 % | 0.88 [ 0.63, 1.22 ] |
| **Subtotal (95% CI)** | **2721** | **2721** | | **4.8 %** | **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)** | **21960** | **18572** | | **100.0 %** | **1.02 [ 0.95, 1.09 ]** |
| Total events: 1707 (Treatment), 1219 (Control) | | | | | |
| Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 2.66$, df = 6 (P = 0.85); $I^2$ =0.0% | | | | | |
| Test for overall effect: Z = 0.43 (P = 0.67) | | | | | |
| Test for subgroup differences: $Chi^2 = 0.79$, df = 1 (P = 0.37), $I^2$ =0.0% | | | | | |



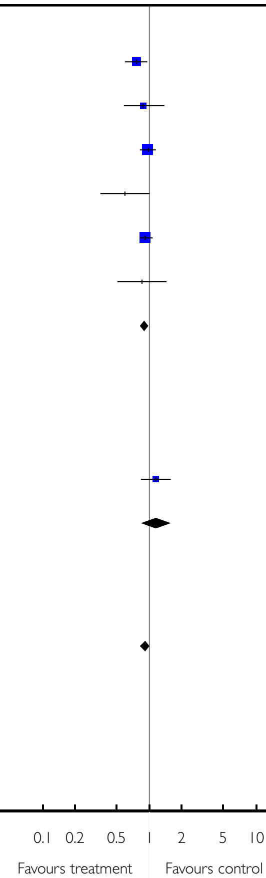
## Analysis 2.2. Comparison 2 Homocysteine-lowering treatment versus other (Sensitivity analysis), Outcome 2 Stroke.

Review: Homocysteine-lowering interventions for preventing cardiovascular events

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

Outcome: 2 Stroke

| Study or subgroup | Treatment n/N | Control n/N | Weight | Risk Ratio M-H,Random,95% CI |
|---|---|---|---|---|
| 1 Trials with low risk of bias (mixed populations) | | | | |
| HOPE-2 2006 | 111/2758 | 147/2764 | 15.6 % | 0.76 [ 0.59, 0.96 ] |
| NORVIT 2006 | 71/2806 | 27/943 | 5.6 % | 0.88 [ 0.57, 1.37 ] |
| SEARCH 2010 | 277/6033 | 286/6031 | 27.7 % | 0.97 [ 0.82, 1.14 ] |
| SU.FOL.OM3 2010 | 21/1242 | 36/1259 | 3.8 % | 0.59 [ 0.35, 1.01 ] |
| VITATOPS 2010 | 360/4089 | 388/4075 | 33.6 % | 0.92 [ 0.81, 1.06 ] |
| WENBIT 2008 | 48/2311 | 19/779 | 3.9 % | 0.85 [ 0.50, 1.44 ] |
| **Subtotal (95% CI)** | **19239** | **15851** | **90.2 %** | **0.89 [ 0.81, 0.98 ]** |
| Total events: 888 (Treatment), 903 (Control) | | | | |
| Heterogeneity: Tau$^2$ = 0.00; Chi$^2$ = 5.36, df = 5 (P = 0.37); I$^2$ =7% | | | | |
| Test for overall effect: Z = 2.29 (P = 0.022) | | | | |
| 2 Trials with low risk of bias (only women included) | | | | |
| WAFACS 2008 | 79/2721 | 69/2721 | 9.8 % | 1.14 [ 0.83, 1.57 ] |
| **Subtotal (95% CI)** | **2721** | **2721** | **9.8 %** | **1.14 [ 0.83, 1.57 ]** |
| Total events: 79 (Treatment), 69 (Control) | | | | |
| Heterogeneity: not applicable | | | | |
| Test for overall effect: Z = 0.83 (P = 0.41) | | | | |
| **Total (95% CI)** | **21960** | **18572** | **100.0 %** | **0.91 [ 0.81, 1.01 ]** |
| Total events: 967 (Treatment), 972 (Control) | | | | |
| Heterogeneity: Tau$^2$ = 0.00; Chi$^2$ = 7.46, df = 6 (P = 0.28); I$^2$ =20% | | | | |
| Test for overall effect: Z = 1.81 (P = 0.070) | | | | |
| Test for subgroup differences: Chi$^2$ = 2.16, df = 1 (P = 0.14), I$^2$ =54% | | | | |



## Analysis 2.3. Comparison 2 Homocysteine-lowering treatment versus other (Sensitivity analysis), Outcome 3 First unstable angina pectoris episode requiring hospitalisation.

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 hospitalisation

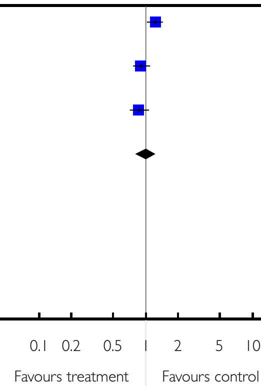
| Study or subgroup | Treatment n/N | Control n/N | Risk Ratio M-H,Random,95% CI | Weight | Risk Ratio M-H,Random,95% CI |
|---|---|---|---|---|---|
| HOPE-2 2006 | 268/2758 | 219/2764 | | 34.7 % | 1.23 [ 1.03, 1.45 ] |
| NORVIT 2006 | 356/2806 | 132/943 | | 33.5 % | 0.91 [ 0.75, 1.09 ] |
| WENBIT 2008 | 280/2311 | 109/779 | | 31.8 % | 0.87 [ 0.70, 1.06 ] |
| **Total (95% CI)** | **7875** | **4486** | | **100.0 %** | **0.99 [ 0.79, 1.24 ]** |

Total events: 904 (Treatment), 460 (Control)

Heterogeneity: $Tau^2 = 0.03$; $Chi^2 = 8.45$, df = 2 (P = 0.01); $I^2 = 76\%$

Test for overall effect: Z = 0.07 (P = 0.95)

Test for subgroup differences: Not applicable



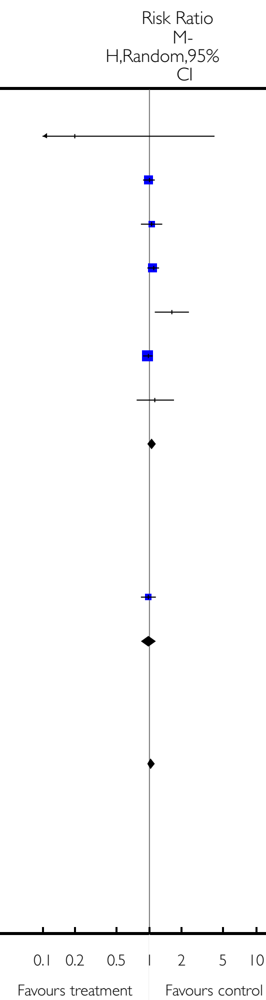
## Analysis 2.4. Comparison 2 Homocysteine-lowering treatment versus other (Sensitivity analysis), Outcome 4 Death from any cause.

Review: Homocysteine-lowering interventions for preventing cardiovascular events

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

Outcome: 4 Death from any cause

| Study or subgroup | Treatment n/N | Control n/N | Weight | Risk Ratio M-H,Random,95% CI |
|---|---|---|---|---|
| 1 Trials with low risk of bias (mixed populations) | | | | |
| BVAIT 2009 | 0/248 | 2/242 | 0.1 % | 0.20 [ 0.01, 4.04 ] |
| HOPE-2 2006 | 470/2758 | 475/2764 | 22.3 % | 0.99 [ 0.88, 1.11 ] |
| NORVIT 2006 | 276/2806 | 89/943 | 9.5 % | 1.04 [ 0.83, 1.31 ] |
| SEARCH 2010 | 463/6033 | 423/6031 | 20.4 % | 1.09 [ 0.96, 1.24 ] |
| SU.FOL.OM3 2010 | 72/1242 | 45/1259 | 4.3 % | 1.62 [ 1.13, 2.33 ] |
| VITATOPS 2010 | 614/4089 | 633/4075 | 25.0 % | 0.97 [ 0.87, 1.07 ] |
| WENBIT 2008 | 101/2311 | 30/779 | 3.6 % | 1.13 [ 0.76, 1.69 ] |
| **Subtotal (95% CI)** | **19487** | **16093** | **85.1 %** | **1.05 [ 0.95, 1.15 ]** |
| Total events: 1996 (Treatment), 1697 (Control) | | | | |
| Heterogeneity: Tau$^2$ = 0.01; Chi$^2$ = 10.13, df = 6 (P = 0.12); I$^2$ =41% | | | | |
| Test for overall effect: Z = 0.98 (P = 0.33) | | | | |
| 2 Trials with low risk of bias (only women included) | | | | |
| WAFACS 2008 | 250/2721 | 256/2721 | 14.9 % | 0.98 [ 0.83, 1.15 ] |
| **Subtotal (95% CI)** | **2721** | **2721** | **14.9 %** | **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)** | **22208** | **18814** | **100.0 %** | **1.03 [ 0.95, 1.12 ]** |
| Total events: 2246 (Treatment), 1953 (Control) | | | | |
| Heterogeneity: Tau$^2$ = 0.00; Chi$^2$ = 10.42, df = 7 (P = 0.17); I$^2$ =33% | | | | |
| Test for overall effect: Z = 0.80 (P = 0.42) | | | | |
| Test for subgroup differences: Chi$^2$ = 0.53, df = 1 (P = 0.47), I$^2$ =0.0% | | | | |



# APPENDICES

## Appendix 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,<br>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 |

## Appendix 2. Search strategies 2008

### CENTRAL

#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 ENSAYO CLINICO CONTROLADO OR Mh ENSAYOS CONTROLADOS ALEATORIOS OR Mh DISTRIBUCIÓN ALEATORIA OR Mh METODO DOBLE CIEGO OR Mh METODO SIMPLE-CIEGO 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

1 exp Vitamin B Complex/
2 vitamin b.tw.
3 folic acid.tw.
4 folate$.tw.
5 ((homocystein$ or homocystin$) adj3 (low$ or reduc$)).tw.
6 pyridoxin$.tw.
7 cobalamin$.tw.
8 cyanocobalamin$.tw.
9 pyridoxol$.tw.
10 Vitamins/
11 or/1-10
12 Cardiovascular Diseases/
13 exp Myocardial Ischemia/
14 exp Brain Ischemia/
15 Cerebrovascular Disorders/
16 (coronary adj3 disease$).tw.
17 angina.tw.
18 myocardial infarct$.tw.
19 heart infarct$.tw.
20 heart attack$.tw.

21 (stroke or strokes).tw.
22 (cerebr$ adj3 (accident$ or infarct$)).tw.
23 (brain adj3 infarct$).tw.
24 apoplexy.tw.
25 (cardiovascular adj2 (disease$ or event$)).tw.
26 Hyperhomocysteinemia/
27 hyperhomocyst?in?emi$.tw.
28 or/12-27
29 11 and 28
30 randomized controlled trial.pt.
31 controlled clinical trial.pt.
32 Randomized controlled trials/
33 random allocation/
34 double blind method/
35 single-blind method/
36 or/30-35
37 exp animal/ not humans/
38 36 not 37
39 clinical trial.pt.
40 exp Clinical Trials as Topic/
41 (clin$ adj25 trial$).ti,ab.
42 ((singl$ or doubl$ or trebl$ or tripl$) adj (blind$ or mask$)).ti,ab.
43 placebos/
44 placebo$.ti,ab.
45 random$.ti,ab.
46 research design/
47 or/39-46
48 47 not 37
49 38 or 48
50 49 and 29

**EMBASE**

1 exp Vitamin B Group/
2 vitamin b.tw.
3 folic acid.tw.
4 folate$.tw.
5 ((homocystein$ or homocystin$) adj3 (low$ or reduc$)).tw.
6 pyridoxin$.tw.
7 cobalamin$.tw.
8 cyanocobalamin$.tw.
9 pyridoxol$.tw.
10 Vitamins/
11 or/1-10
12 Cardiovascular Diseases/
13 exp ischaemic heart disease/
14 exp Coronary Artery Disease/
15 exp Brain Ischemia/
16 cerebrovascular disease/
17 stroke/
18 cerebrovascular accident/
19 (coronary adj3 disease$).tw.
20 angina.tw.

21 myocardial infarct$.tw.
22 heart infarct$.tw.
23 heart attack$.tw.
24 (stroke or strokes).tw.
25 (cerebr$ adj3 (accident$ or infarct$)).tw.
26 (brain adj3 infarct$).tw.
27 apoplexy.tw.
28 (cardiovascular adj2 (disease$ or event$)).tw.
29 Hyperhomocysteinemia/
30 hyperhomocyst?in?emi$.tw.
31 or/12-30
32 11 and 31
33 controlled clinical trial/
34 random$.tw.
35 randomized controlled trial/
36 follow-up.tw.
37 double blind procedure/
38 placebo$.tw.
39 placebo/
40 factorial$.ti,ab.
41 (crossover$ or cross-over$).ti,ab.
42 (double$ adj blind$).ti,ab.
43 (singl$ adj blind$).ti,ab.
44 assign$.ti,ab.
45 allocat$.ti,ab.
46 volunteer$.ti,ab.
47 Crossover Procedure/
48 Single Blind Procedure/
49 or/33-48
50 32 and 49

### 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")

## Appendix 3. Search strategies 2012

### CENTRAL

#1 MeSH descriptor Vitamin B Complex explode all trees
#2 (vitamin b)
#3 folic acid
#4 folate*
#5 ((homocystein* or homocystin*) near/3 (low* or reduc*))
#6 (pyridoxin*)
#7 (cobalamin*)
#8 (cyanocobalamin*)
#9 (pyridoxol*)
#10 MeSH descriptor Vitamins, this term only
#11 (#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10)
#12 MeSH descriptor Cardiovascular Diseases, this term only
#13 MeSH descriptor Myocardial Ischemia explode all trees
#14 MeSH descriptor Brain Ischemia explode all trees
#15 MeSH descriptor Cerebrovascular Disorders, this term only
#16 (coronary near/3 disease*)
#17 (angina)
#18 (myocardial infarct*)
#19 (heart infarct*)
#20 (heart attack*)
#21 (stroke or strokes)
#22 (cerebr* near/3 (accident* or infarct*))
#23 (brain near/3 infarct*)
#24 (apoplexy)
#25 (cardiovascular near/2 (disease* or event*))
#26 MeSH descriptor Hyperhomocysteinemia, this term only
#27 hyperhomocyst?in?emi*
#28 (#12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27)
#29 (#11 AND #28)

### MEDLINE

1 exp Vitamin B Complex/
2 vitamin b.tw.
3 folic acid.tw.
4 folate$.tw.
5 ((homocystein$ or homocystin$) adj3 (low$ or reduc$)).tw.
6 pyridoxin$.tw.
7 cobalamin$.tw.
8 cyanocobalamin$.tw.
9 pyridoxol$.tw.
10 Vitamins/
11 or/1-10
12 Cardiovascular Diseases/
13 exp Myocardial Ischemia/
14 exp Brain Ischemia/
15 Cerebrovascular Disorders/
16 (coronary adj3 disease$).tw.

17 angina.tw.
18 myocardial infarct$.tw.
19 heart infarct$.tw.
20 heart attack$.tw.
21 (stroke or strokes).tw.
22 (cerebr$ adj3 (accident$ or infarct$)).tw.
23 (brain adj3 infarct$).tw.
24 apoplexy.tw.
25 (cardiovascular adj2 (disease$ or event$)).tw.
26 Hyperhomocysteinemia/
27 hyperhomocyst?in?emi$.tw.
28 or/12-27
29 11 and 28
30 randomized controlled trial.pt.
31 controlled clinical trial.pt.
32 randomized.ab.
33 placebo.ab.
34 drug therapy.fs.
35 randomly.ab.
36 trial.ab.
37 groups.ab.
38 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37
39 exp animals/ not humans.sh. (3663238)
40 38 not 39
41 29 and 40
42 (200808* or 200809* or 20081* or 2009* or 2010* or 2011* or 2012*).ed.
43 41 and 42

### EMBASE

1 exp Vitamin B Complex/
2 vitamin b.tw.
3 folic acid.tw.
4 folate$.tw.
5 ((homocystein$ or homocystin$) adj3 (low$ or reduc$)).tw.
6 pyridoxin$.tw.
7 cobalamin$.tw.
8 cyanocobalamin$.tw.
9 pyridoxol$.tw.
10 Vitamins/
11 or/1-10
12 Cardiovascular Diseases/
13 exp Myocardial Ischemia/
14 exp Brain Ischemia/
15 Cerebrovascular Disorders/
16 (coronary adj3 disease$).tw.
17 angina.tw.
18 myocardial infarct$.tw.
19 heart infarct$.tw.
20 heart attack$.tw.
21 (stroke or strokes).tw.
22 (cerebr$ adj3 (accident$ or infarct$)).tw.
23 (brain adj3 infarct$).tw.

24 apoplexy.tw.
25 (cardiovascular adj2 (disease$ or event$)).tw.
26 Hyperhomocysteinemia/
27 hyperhomocyst?in?emi$.tw.
28 or/12-27
29 11 and 28
30 random$.tw.
31 factorial$.tw.
32 crossover$.tw.
33 cross over$.tw.
34 cross-over$.tw.
35 placebo$.tw.
36 (doubl$ adj blind$).tw.
37 (singl$ adj blind$).tw.
38 assign$.tw.
39 allocat$.tw.
40 volunteer$.tw.
41 crossover procedure/
42 double blind procedure/
43 randomized controlled trial/
44 single blind procedure/
45 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44
46 (animal/ or nonhuman/) not human/
47 45 not 46
48 29 and 47
49 (200808* or 200809* or 20081* or 2009* or 2010* or 2011* or 2012*).dd.
50 48 and 49

### Web of Science

#24 #23 AND #22
#23 Topic=((random* or blind* or allocat* or assign* or trial* or placebo* or crossover* or cross-over*))
#22 #21 AND #9
#21 #20 OR #19 OR #18 OR #17 OR #16 OR #15 OR #14 OR #13 OR #12 OR #11
#20 Topic=(hyperhomocyst$in$emi*)
#19 Topic=((cardiovascular near/2 (disease* or event*)))
#18 Topic=(apoplexy)
#17 Topic=((brain near/3 infarct*))
#16 Topic=((cerebr* near/3 (accident* or infarct*)))
#15 Topic=((stroke or strokes))
#14 Topic=(heart attack*)
#13 Topic=(heart infarct*)
#12 Topic=(myocardial infarct*)
#11 Topic=(angina)
#10 Topic=((coronary near/3 disease*))
#9 #8 OR #7 OR #6 OR #5 OR #4 OR #3 OR #2 OR #1
#8 Topic=(pyridoxol*)
#7 Topic=(cyanocobalamin*)
#6 Topic=(cobalamin*)
#5 Topic=(pyridoxin*)
#4 Topic=(((homocystein*) near/3 (low$ or reduc*))) OR Topic=(((homocystin*) near/3 (low or reduc*)))
#3 Topic=(folate*)
#2 Topic=(“folic acid”)

#1 Topic=("vitamin b")

## Appendix 4. Search strategies 2014

### CENTRAL

#1 MeSH descriptor Vitamin B Complex explode all trees
#2 (vitamin b)
#3 folic acid
#4 folate*
#5 ((homocystein* or homocystin*) near/3 (low* or reduc*))
#6 (pyridoxin*)
#7 (cobalamin*)
#8 (cyanocobalamin*)
#9 (pyridoxol*)
#10 MeSH descriptor Vitamins, this term only
#11 (#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10)
#12 MeSH descriptor Cardiovascular Diseases, this term only
#13 MeSH descriptor Myocardial Ischemia explode all trees
#14 MeSH descriptor Brain Ischemia explode all trees
#15 MeSH descriptor Cerebrovascular Disorders, this term only
#16 (coronary near/3 disease*)
#17 (angina)
#18 (myocardial infarct*)
#19 (heart infarct*)
#20 (heart attack*)
#21 (stroke or strokes)
#22 (cerebr* near/3 (accident* or infarct*))
#23 (brain near/3 infarct*)
#24 (apoplexy)
#25 (cardiovascular near/2 (disease* or event*))
#26 MeSH descriptor Hyperhomocysteinemia, this term only
#27 hyperhomocyst?in?emi*
#28 (#12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27)
#29 (#11 AND #28)

### MEDLINE

1 exp Vitamin B Complex/
2 vitamin b.tw.
3 folic acid.tw.
4 folate$.tw.
5 ((homocystein$ or homocystin$) adj3 (low$ or reduc$)).tw.
6 pyridoxin$.tw.
7 cobalamin$.tw.
8 cyanocobalamin$.tw.
9 pyridoxol$.tw.
10 Vitamins/
11 or/1-10
12 Cardiovascular Diseases/

13 exp Myocardial Ischemia/
14 exp Brain Ischemia/
15 Cerebrovascular Disorders/
16 (coronary adj3 disease$).tw.
17 angina.tw.
18 myocardial infarct$.tw.
19 heart infarct$.tw.
20 heart attack$.tw.
21 (stroke or strokes).tw.
22 (cerebr$ adj3 (accident$ or infarct$)).tw.
23 (brain adj3 infarct$).tw.
24 apoplexy.tw.
25 (cardiovascular adj2 (disease$ or event$)).tw.
26 Hyperhomocysteinemia/
27 hyperhomocyst?in?emi$.tw.
28 or/12-27
29 11 and 28
30 randomized controlled trial.pt.
31 controlled clinical trial.pt.
32 randomized.ab.
33 placebo.ab.
34 drug therapy.fs.
35 randomly.ab.
36 trial.ab.
37 groups.ab.
38 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37
39 exp animals/ not humans.sh. (3663238)
40 38 not 39
41 29 and 40
42 (2012* or 2013* or 2014*).ed.
43 41 and 42

### EMBASE

1 exp Vitamin B Complex/
2 vitamin b.tw.
3 folic acid.tw.
4 folate$.tw.
5 ((homocystein$ or homocystin$) adj3 (low$ or reduc$)).tw.
6 pyridoxin$.tw.
7 cobalamin$.tw.
8 cyanocobalamin$.tw.
9 pyridoxol$.tw.
10 Vitamins/
11 or/1-10
12 Cardiovascular Diseases/
13 exp Myocardial Ischemia/
14 exp Brain Ischemia/
15 Cerebrovascular Disorders/
16 (coronary adj3 disease$).tw.
17 angina.tw.
18 myocardial infarct$.tw.
19 heart infarct$.tw.

20 heart attack$.tw.
21 (stroke or strokes).tw.
22 (cerebr$ adj3 (accident$ or infarct$)).tw.
23 (brain adj3 infarct$).tw.
24 apoplexy.tw.
25 (cardiovascular adj2 (disease$ or event$)).tw.
26 Hyperhomocysteinemia/
27 hyperhomocyst?in?emi$.tw.
28 or/12-27
29 11 and 28
30 random$.tw.
31 factorial$.tw.
32 crossover$.tw.
33 cross over$.tw.
34 cross-over$.tw.
35 placebo$.tw.
36 (doubl$ adj blind$).tw.
37 (singl$ adj blind$).tw.
38 assign$.tw.
39 allocat$.tw.
40 volunteer$.tw.
41 crossover procedure/
42 double blind procedure/
43 randomized controlled trial/
44 single blind procedure/
45 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44
46 (animal/ or nonhuman/) not human/
47 45 not 46
48 29 and 47
49 (2012* or 2013* or 2014*).dd.
50 48 and 49

### Web of Science

#24 #23 AND #22
#23 Topic=((random* or blind* or allocat* or assign* or trial* or placebo* or crossover* or cross-over*))
#22 #21 AND #9
#21 #20 OR #19 OR #18 OR #17 OR #16 OR #15 OR #14 OR #13 OR #12 OR #11
#20 Topic=(hyperhomocyst$in$emi*)
#19 Topic=((cardiovascular near/2 (disease* or event*)))
#18 Topic=(apoplexy)
#17 Topic=((brain near/3 infarct*))
#16 Topic=((cerebr* near/3 (accident* or infarct*)))
#15 Topic=((stroke or strokes))
#14 Topic=(heart attack*)
#13 Topic=(heart infarct*)
#12 Topic=(myocardial infarct*)
#11 Topic=(angina)
#10 Topic=((coronary near/3 disease*))
#9 #8 OR #7 OR #6 OR #5 OR #4 OR #3 OR #2 OR #1
#8 Topic=(pyridoxol*)
#7 Topic=(cyanocobalamin*)
#6 Topic=(cobalamin*)

#5 Topic=(pyridoxin*)
#4 Topic=(((homocystein*) near/3 (low$ or reduc*))) OR Topic=(((homocystin*) near/3 (low or reduc*)))
#3 Topic=(folate*)
#2 Topic=("folic acid")
#1 Topic=("vitamin b")

## Appendix 5. Definitions of myocardial infarction (MI), stroke, unstable angina and death

| Trial | Myocardial infarction | Stroke | Death | | |
|---|---|---|---|---|---|
| BVAIT 2009 | Not available | Not available | Not available | | |
| HOPE-2 2006 | 2 of the following 3 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 haemorrhagic). 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 post-mortem 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.<br>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 |

(*Continued*)

| | | | | | |
|---|---|---|---|---|---|
| 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 too long to summarise in this table |
| SEARCH 2010 | https://www.ctsu.ox.ac.uk/research/research-archive/searchs/search-study-protocol/view Accessed: 7 January 2015 | https://www.ctsu.ox.ac.uk/research/research-archive/searchs/search-study-protocol/view Accessed: 7 January 2015 | https://www.ctsu.ox.ac.uk/research/research-archive/searchs/search-study-protocol/view Accessed: 7 January 2015 | https://www.ctsu.ox.ac.uk/research/research-archive/searchs/search-study-protocol/view Accessed: 7 January 2015 | Definitions are too long to summarise in this table |
| SU.FOL.OM3 2010 | Myocardial infarction (ICD-10 (International Classification of Diseases, 10th revision) codes I21.0-I21.9) was defined on the basis of 2 or more of the criteria: typical chest pain, electrocardiographic changes consistent with myocardial infarction and cardiac enzyme increase | An acute cerebral ischaemic event was defined as an ischaemic cerebrovascular accident based on clinical criteria confirmed by computed tomography or magnetic resonance imaging and a Rankin score 3 at inclusion (ICD-10 codes I63.0-I63.9) | Acute coronary syndrome without myocardial infarction (ICD-10 codes I20.0-I20.1) was initially defined by the presence of 3 criteria: typical chest pain, electrocardiographic changes consistent with coronary artery disease without myocardial infarction and evidence of coronary artery disease (myocardial infarction, angina with angiographic evidence of stenosis > 50% in one or more coronary arteries, or angina pectoris corroborated by coronary angiography or exercise testing, or coronary angioplasty or coronary artery bypass graft procedure). Suspected acute coronary syndrome without characteris- | | |

(*Continued*)

| | | | | | |
|---|---|---|---|---|---|
| | | | tic electrocardiographic evidence of myocardial infarction provided there was angiographic evidence of coronary artery disease | | |
| 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 Organization criteria | A new neurologic deficit of sudden onset that persisted for more than 24 hours or until death within 24 hours | Not available | Death 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 endpoint committee on the basis of a death certificate | |

(*Continued*)

| | | | | | |
|---|---|---|---|---|---|
| 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 | |

## WHAT'S NEW

Last assessed as up-to-date: 12 February 2014.

| Date | Event | Description |
|---|---|---|
| 15 October 2014 | New citation required but conclusions have not changed | We found no new trials for inclusion. |
| 9 July 2014 | New search has been performed | We updated the searches to February 2014.<br>This updated Cochrane Review now has only three authors. |

## HISTORY

Protocol first published: Issue 3, 2007

Review first published: Issue 4, 2009

| Date | Event | Description |
|---|---|---|
| 7 March 2012 | New citation required but conclusions have not changed | This new updated version includes four additional RCTs and the conclusions are not changed |
| 21 February 2012 | New search has been performed | We updated the searches to 21 February 2012. |

# CONTRIBUTIONS OF AUTHORS

Arturo Marti-Carvajal took the lead on writing up the Cochrane Review.

Ivan Solà identified trials, extracted data, edited the 'Summary of findings' table and drafted the Cochrane Review.

Dimitris Lathyris extracted and checked the data and reviewed the Cochrane Review.

# DECLARATIONS OF INTEREST

Ivan Solà and Dimitrios Lathyris: none known.

In 2004 Arturo Martí-Carvajal was employed by Eli Lilly to run a four-hour workshop on 'How to critically appraise clinical trials on osteoporosis and how to teach this'. This activity was not related to his work with The Cochrane Collaboration or any Cochrane Review.

In 2007 Arturo Martí-Carvajal was employed by Merck to run a four-hour workshop 'How to critically appraise clinical trials and how to teach this'. This activity was not related to his work with The Cochrane Collaboration or any Cochrane Review.


# SOURCES OF SUPPORT

## Internal sources

- No sources of support supplied

## External sources

- Iberoamerican Cochrane Centre, Spain.

Academic

- Cochrane Heart Group, UK.

Academic


# DIFFERENCES BETWEEN PROTOCOL AND REVIEW

This update includes a trial sequential analysis.

In the first version of the review (Marti-Carvajal 2009), we searched the Allied and Complementary Medicine - AMED database (accessed through Ovid) and the Cochrane Stroke Group Specialised Register. For this update, we did not search either database.

This update includes the Plain Summary Language 'section' adapted according to the new recommendations of The Cochrane Collaboration. The results include the quality of the evidence assessed according to GRADE ('Summary of findings').

## I N D E X   T E R M S

### Medical Subject Headings (MeSH)

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

### MeSH check words

Humans