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Homocysteine-lowering interventions for preventing cardiovascular events (Review)

Martí-Carvajal AJ, Solà I, Lathyris D, Dayer M

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[Intervention Review]

Homocysteine-lowering interventions for preventing cardiovascular events

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ABSTRACT

Background

Cardiovascular disease, which includes coronary artery disease, stroke and peripheral vascular disease, is a leading cause of death worldwide. Homocysteine is an amino acid with biological functions in methionine metabolism. A postulated risk factor for cardiovascular disease is an elevated circulating total homocysteine level. The impact of homocysteine-lowering interventions, given to patients in the form of vitamins B6, B9 or B12 supplements, on cardiovascular events has been investigated. This is an update of a review previously published in 2009, 2013, and 2015.

Objectives

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

Search methods

We searched the Cochrane Central Register of Controlled Trials (CENTRAL 2017, Issue 5), MEDLINE (1946 to 1 June 2017), Embase (1980 to 2017 week 22) and LILACS (1986 to 1 June 2017). We also searched Web of Science (1970 to 1 June 2017). 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 calculated the number needed to treat for an additional beneficial outcome (NNTB). We measured statistical heterogeneity using the I^2 statistic. We used a random-effects model. We conducted trial sequential analyses, Bayes factor, and fragility indices where appropriate.

Main results

In this third update, we identified three new randomised controlled trials, for a total of 15 randomised controlled trials involving 71,422 participants. Nine trials (60%) had low risk of bias, length of follow-up ranged from one to 7.3 years. Compared with placebo, there were no differences in effects of homocysteine-lowering interventions on myocardial infarction (homocysteine-lowering = 7.1% versus placebo = 6.0%; RR 1.02, 95% confidence interval (CI) 0.95 to 1.10, I^2 = 0%, 12 trials; N = 46,699; Bayes factor 1.04, high-quality evidence), death from any cause (homocysteine-lowering = 11.7% versus placebo = 12.3%, RR 1.01, 95% CI 0.96 to 1.06, I^2 = 0%, 11 trials, N = 44,817; Bayes factor = 1.05, high-quality evidence), or serious adverse events (homocysteine-lowering = 8.3% versus comparator = 8.5%, RR 1.07, 95% CI 1.00 to 1.14, I^2 = 0%, eight trials, N = 35,788; high-quality evidence). Compared with placebo, homocysteine-lowering interventions were associated with reduced stroke outcome (homocysteine-lowering = 4.3% versus comparator = 5.1%, RR 0.90, 95% CI 0.82 to 0.99, I^2 = 8%, 10 trials, N = 44,224; high-quality evidence). Compared with low doses, there were uncertain effects of high doses of homocysteine-lowering interventions on stroke (high = 10.8% versus low = 11.2%, RR 0.90, 95% CI 0.66 to 1.22, I^2 = 72%, two trials, N = 3929; very low-quality evidence).

We found no evidence of publication bias.

Authors' conclusions

In this third update of the Cochrane review, there were no differences in effects of homocysteine-lowering interventions in the form of supplements of vitamins B6, B9 or B12 given alone or in combination comparing with placebo on myocardial infarction, death from any cause or adverse events. In terms of stroke, this review found a small difference in effect favouring to homocysteine-lowering interventions in the form of supplements of vitamins B6, B9 or B12 given alone or in combination comparing with placebo.

There were uncertain effects of enalapril plus folic acid compared with enalapril on stroke; approximately 143 (95% CI 85 to 428) people would need to be treated for 5.4 years to prevent 1 stroke, this evidence emerged from one mega-trial.

Trial sequential analyses showed that additional trials are unlikely to increase the certainty about the findings of this issue regarding homocysteine-lowering interventions versus placebo. There is a need for additional trials comparing homocysteine-lowering interventions combined with antihypertensive medication versus antihypertensive medication, and homocysteine-lowering interventions at high doses versus homocysteine-lowering interventions at low doses. Potential trials should be large and co-operative.

PLAIN LANGUAGE SUMMARY

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

Review question
We reviewed whether particular vitamins, which lower homocysteine, prevent cardiovascular events such as heart attack and stroke.

Background
Cardiovascular disease, which includes heart attacks and strokes, is the number one cause of death worldwide. Many people with cardiovascular disease may not have symptoms, but be at high risk. Diabetes mellitus, high blood pressure, smoking and a high cholesterol, as well as a family history of cardiovascular disease are well known risk factors. Elevated total homocysteine levels have recently been identified as a risk factor for cardiovascular disease. Homocysteine is an amino acid, its levels in the blood are influenced by blood levels of B vitamins: cyanocobalamin (B12), folic acid (B9) and pyridoxine (B6). This report is an update from a previous review published in 2015.

Study characteristics
The evidence is current to June 2017. We included 15 studies involving 71,422 participants living in countries with or without mandatory supplementation of foods with vitamins. These studies compared different regimens of B vitamins (cyanocobalamin (B12), folic acid (B9) and pyridoxine (B6)) with a control or any other comparison group. The studies were published between 2002 and 2015.

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, prevented heart attack or reduced death rates in participants at risk of, or living with cardiovascular disease. Homocysteine-lowering interventions combined with antihypertensive medication had uncertain effects on stroke, approximately 143 people would need to be treated for 5.4 years to prevent 1 stroke. Homocysteine-lowering interventions compared with placebo or any other comparison did not affect serious adverse events (cancer).

Quality of evidence
The quality of evidence from these studies was generally high.

Summary of findings 1. Homocysteine-lowering interventions (Vitamin B6 (pyridoxine; pyridoxal); B9 (folic acid) or B12 (cyanocobalamin) compared with placebo or standard care for preventing cardiovascular events

Homocysteine-lowering interventions (vitamins B6 (pyridoxine; pyridoxal); B9 (folic acid) or B12 (cyanocobalamin) 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 (vitamins B6 (pyridoxine; pyridoxal), B9 (folic acid) or B12 (cyanocobalamin).
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 (vitamins B6 (pyridoxine; pyridoxal); B9 (folic acid) or B12 (cyanocobalamin)				
Myocardial infarction Follow-up: 1 to 7.3 years	**60 per 1000**	**61 per 1000** (57 to 66)	**RR 1.02** (0.95 to 1.10)	46,699 (12 trials)	⊕⊕⊕⊕ **high**	
Stroke Follow-up: 1 to 7.3 years	**51 per 1000**	**46 per 1000** (42 to 50)	**RR 0.90** (0.82 to 0.99)	44,224 (10 trials)	⊕⊕⊕⊕ **high**	
Death by any cause Follow-up: 1 to 7.3 years	**123 per 1000**	**124 per 1000** (118 to 130)	**RR 1.01** (0.96 to 1.06)	44,817 (11 trials)	⊕⊕⊕⊕ **high**	
Adverse events Follow-up: 3.4 to 7.3 years	**85 per 1000**	**91 per 1000** (85 to 97)	**RR 1.07** (1.00 to 1.14)	35,788 (8 trials)	⊕⊕⊕⊕ **high**	Cancer is the only reported adverse event.

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is the outcomes of the study control arms. 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.

Summary of findings 2. Homocysteine-lowering interventions (high dose) compared with homocysteine-lowering interventions (low dose) for preventing cardiovascular events

Homocysteine-lowering interventions (high dose) compared with homocysteine lowering interventions (low dose) for preventing cardiovascular events

Patient or population: adults at risk of or with established cardiovascular disease
Settings: outpatients
Intervention: homocysteine-lowering interventions (high dose) either (folic acid; vitamin B12 (cyanocobalamin) and vitamin B6 (pyridoxine; pyridoxal) or folic acid
Comparison: homocysteine-lowering interventions (low dose) either (folic acid; vitamin B12; vitamin B6 per day) or folic acid

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Homocysteine-lowering interventions (low-dose)	Homocysteine-lowering interventions (high-dose)				
Myocardial infarction Follow-up: 2 years	**44 per 1000**	**40 per 1000** (29 to 54)	**RR 0.90** (0.66 to 1.23)	3649 (1 trial)	⊕⊕⊕⊖ **moderate** [1]	VISP 2004: • High dose (2.5 mg folic acid; 0.4 mg vitamin B12 (cyanocobalamin) and 25 mg vitamin B6 (pyridoxine; pyridoxal) • Low dose (20 micrograms folic acid; 6 micrograms vitamin B12; 200 micrograms vitamin B6 per day).
Stroke Follow-up: 2 to 5 years	**112 per 1000**	**101 per 1000** (74 to 137)	**RR 0.90** (0.66 to 1.22)	3929 (2 trials)	⊕⊖⊖⊖ **very low** [1, 2, 3]	1. Li 2015a was conducted including only Chinese elderly females.Trial used only folic acid as homocysteine-lowering intervention. • High-dose folic acid (0.8 mg/d) • Low-dose folic acid (0.4 mg/d)) 2. VISP 2004: • High dose (2.5 mg folic acid; 0.4 mg vitamin B12 (cyanocobalamin) and 25 mg vitamin B6 (pyridoxine; pyridoxal) • Low dose (20 micrograms folic acid; 6 micrograms vitamin B12; 200 micrograms vitamin B6 per day).

Death by any cause Follow-up: 2 years	**64 per 1000**	**55 per 1000** (42 to 71)	**RR 0.86** (0.66 to 1.11)	3649 (1 trial)	⊕⊕⊕⊖ **moderate** [1]	VISP 2004: • High dose (2.5 mg folic acid; 0.4 mg vitamin B12 (cyanocobalamin) and 25 mg vitamin B6 (pyridoxine; pyridoxal) • Low dose (20 micrograms folic acid; 6 micrograms vitamin B12; 200 micrograms vitamin B6 per day).
Cancer			Not estimable	-		Li 2015a and VISP 2004 reported no information on this outcome.

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is the outcomes of the study control arms. 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] Downgraded one level for imprecision due to low number of events
[2] Dowgraded one level for risk of bias as one trial (Li 2015a) was rated as having unclear risk of selection, conduction and detection biases
[3] Downgraded one level for heterogeneity (I-squared: 72%).

Summary of findings 3. Enalapril plus folic acid compared with enalapril for adults with hypertension

Enalapril plus folic acid compared with folic acid for adults with hypertension

Patient or population: adults with hypertension
Settings: Chinese outpatients
Intervention: enalapril (10 mg) plus folic acid (0.8 mg)
Comparison: enalapril (10 mg)

Outcomes	**Illustrative comparative risks* (95% CI)**		**Relative effect (95% CI)**	**No of Participants (studies)**	**Quality of the evidence (GRADE)**	**Comments**
	Assumed risk	**Corresponding risk**				
	Folic acid	**Enalapril plus folic acid**				
Myocardial infarction Follow-up: median 4.5 years	**2 per 1000**	**2 per 1000** (1 to 4)	**RR 1.04** (0.60 to 1.82)	20,702 (1 trial)	⊕⊕⊕⊖	

					moderate [1]	
Stroke Follow-up: median 4.5 years	**34 per 1000**	**27 per 1000** (23 to 32)	**RR 0.79** (0.68 to 0.93)	20,702 (1 trial)	⊕⊕⊕⊕ **high**	
First unstable angina pectoris episode requiring hospitalisation			Not estimable	-		CSPPT 2015 did not assess this outcome.
Death from any cause Follow-up: median 4.5 years	**31 per 1000**	**29 per 1000** (25 to 34)	**RR 0.94** (0.81 to 1.10)	20,702 (1 trial)	⊕⊕⊕⊕ **high**	
Serious adverse event (cancer) Follow-up: median 4.5 years	**8 per 1000**	**8 per 1000** (6 to 11)	**RR 0.96** (0.71 to 1.31)	20,243 (1 trial)	⊕⊕⊕⊖ **moderate** [1]	CSPPT 2015 included either neoplasms benign, malignant or unspecified

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is the outcomes of the study control arms. 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]Downgraded one level for imprecision due to low number of events.

BACKGROUND

Description of the condition

The burden of cardiovascular disease

Cardiovascular disease is the number one cause of death worldwide (Barquera 2015; Smith 2012). 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. The most common causes of cardiovascular disease-related morbidity and mortality are ischaemic heart disease and stroke (Li 2016; Maredza 2015; Oliveira 2015; Prabhakaran 2016).

The burden of cardiovascular disease is significant and ischaemic heart disease is the single largest cause of death worldwide (Bansilal 2015; Kwan 2016). Global deaths from cardiovascular disease increased by 41% between 1990 and 2013 (Roth 2015a). It has been pointed out that cardiovascular diseases cause more than 4 million deaths/year in the 53 countries of the World Health Organization European Region and over 1.9 million deaths in the European Union (Bansilal 2015). It has been estimated that there will be 7.8 million premature cardiovascular deaths in 2025 (Roth 2015b).

Cardiovascular diseases account for about one-half of non communicable diseases deaths (Benziger 2016). The majority of cardiovascular disease deaths occur in low- and middle-income countries (Barquera 2015; Benziger 2016; Oliveira 2015; Prabhakaran 2016). The major risk factors for cardiovascular diseases include tobacco use, high blood pressure, high blood glucose, lipid abnormalities, high levels of body mass index and physical inactivity (Barquera 2015; Lackland 2015; Li 2016; Roth 2015b; Singh 2015; Tzoulaki 2016; Yeates 2015).

Homocysteine as a risk factor for cardiovascular disease

In 1962, it was hypothesised that increased levels of total homocysteine may cause vascular disease: the homocysteine theory of arteriosclerosis (McCully 2015a). The pathways through which total homocysteine levels may cause damage to endothelial cells and lead to atherosclerosis have been widely described (Ganguly 2015; McCully 2015b Pushpakumar 2014). It has been pointed out that homocysteine reduces the bioavailability of the nitric oxide, a potent vasodilator (Lai 2015b). Another mechanism would be through an integration of the roles of homocysteine and folic acid in cardiovascular pathobiology, known as methoxistasis (Joseph 2013). The molecular and cellular effect of homocysteine metabolism imbalance yields oxidative stress which is cytotoxic (Skovierova 2016). The cellular status of homocysteine is not correlated with the homocysteine levels in plasma, which may explain the considerable differences that there are between epidemiological, intervention and basic research reports (Hannibal 2016).

Homocysteine is a non-proteinogenic amino acid derived in methionine metabolism (Skovierova 2016). Several observational studies had shown that a raised blood homocysteine level was 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). Currently, there is no evidence to support cardiovascular risk reduction by homocysteine-lowering interventions (Cybulska 2015; Li 2015; Martí-Carvajal 2009; Martí-Carvajal 2013; Martí-Carvajal 2015 (three previous versions of this review)). The American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Goff 2014) and The European Guidelines on cardiovascular disease prevention in clinical practice (Perk 2012) ratify that homocysteine is not a causal risk factor for cardiovascular disease.

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 (Mudd 2000). However, there is some controversy about the definition of the degree of hyperhomocysteinaemia. Fasting total homocysteine level concentrations between 12 µmol/L and 30 µmol/L are termed mild or moderate, while intermediate hyperhomocysteinaemia includes levels between 31 µmol/L 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).

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), given as a supplement.

How the intervention might work

The B-complex vitamins are essential for homocysteine metabolism; they are involved in both the transformation and excretion pathways of homocysteine (McCully 2015a; McCully 2015b). 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 a methionine load, which occurs in experimental situations. However, at least two studies have shown the contrary (Gori 2007; Sofi 2008). It is, as a result, believed to be a weak determinant of circulating total homocysteine levels.

Why it is important to do this review

This is the third update of this Cochrane review and has been performed to identify and review the latest evidence.

OBJECTIVES

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

1. are effective in preventing cardiovascular events and/or all-cause mortality;
2. are safe;
3. differ in efficacy or safety.

METHODS

Criteria for considering studies for this review

Types of studies

Randomised controlled trials (RCTs) 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 (pyridoxine; pyridoxal), B9 (folic acid) or B12 (cyanocobalamin) 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) versus standard treatment alone.

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 1), 2012 (Appendix 2), and 2014 (Appendix 3). Search strategies for 2017 are shown in Appendix 4.

We updated the searches of the Cochrane Central Register of Controlled Trials (CENTRAL 2017, Issue 5), MEDLINE OVID (1946 to 1 June 2017), Embase OVID (1980 to 2017 week 22) and Web of Science (Thomson Reuters, 1970 to 1 June 2017). The search of LILACS was last run on 1 June 2017. In a previous version (Martí-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) and ClinicalTrials.gov (https://clinicaltrials.gov/).

We also searched websites of U.S. Food and Drug Administration (www.fda.gov) and European Medicines Agency (www.ema.europa.eu) for unpublished information on homocysteine-lowering interventions.

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

Data collection and analysis

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

Selection of studies

Two authors (AMC and IS) 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 review authors (AMC and IS) 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 review author (DL) to check the data entered into the Review Manager software. Two review authors (AMC and IS) assessed the included studies and entered the information into tables; see Characteristics of included studies.

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.

1. Generation of the allocation sequence
2. Allocation concealment
3. Blinding (or masking)
4. Incomplete outcome data
5. Selective outcome reporting
6. Other bias

See Appendix 5 for details of domains.

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, death from any cause and serious or non-serious adverse events as recommended by Higgins 2011. We calculated the number needed to treat for an additional beneficial outcome (NNTB) if the RR was significant (P value = < 0.05). NNTB is a measure of assessment of clinical useful of the consequences of treatment (Laupacis 1988). We estimated NNTB with GraphPad software.

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 asymmetry in funnel plots for myocardial infarction, stroke and death from any cause, and devoted to detect potential publication bias and other causes of asymmetry (Sterne 2001). We used the contour-enhanced funnel plot for differentiating asymmetry due to publication bias from that due to other factors (Peters 2008). We assessed likelihood of publication bias with Harbord and Peters tests (Sterne 2011a; Sterne 2011b). We used STATA statistical software V.14.0 (StataCorp LP) to perform conventional and contour funnel plots.

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; Wetterslev 2017). 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, and by trials including participants without cardiovascular disease versus trials including participants with cardiovascular disease.

Sensitivity analysis

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

'Summary of findings' tables

We used The Grading of Recommendations Assessment, Development and Evaluation (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). One review author constructed Summary of findings 1; Summary of findings 2; Summary of findings 3 using the GRADEpro software (GRADEpro 2008). We involved a second review author to check the data.

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 (Guyatt 2008; Guyatt 2013).

Fragility Index

We calculated the fragility index (FI) if the RR was significant (P value = < 0.05). FI is a measure to identify the number of events required to change statistically significant results to non-significant results (Walsh 2014). The FI was only applied to RCTs where the allocation 1:1 and to binary data. We estimated the FI with the Fragility Index Calculator.

Bayes Factors

We estimated the threshold for clinical relevance using a Bayes factor (Jakobsen 2014). This is a likelihood ratio indicate the relative strength of evidence for two theories (Dienes 2014; Goodman 1999; Goodman 2005). A Bayes factor is a comparison of how well two hypotheses (the null hypothesis -H0- and the alternative hypothesis -H1-) predict the data (Goodman 1999). A Bayes factor provides a continuous measure of evidence for H1 over H0. When a Bayes factor is 1, the evidence does not favour either model over the other. As a Bayes factor increase above 1 (towards infinity) the evidence favours H1 over H0. As a Bayes factor decreases below 1 (towards 0) the evidence favours H0 over H1 (Dienes 2008; Dienes 2014; Dienes 2017). We used Dienes' Calculator for estimating Bayes factors.

RESULTS

Description of studies

The search in June 2017 identified 1464 records, which resulted in 1073 unique references after duplicates were removed. After examining the titles and abstracts we excluded 1036 references. We obtained full reprints of the remaining 37 references for more detailed examination, of which 22 reports were excluded. The remaining 15 references identified were for three new randomised clinical trials (B-PROOF 2015; CSPPT 2015; Li 2015a), 11 of which related to one of the new trials (CSPPT 2015).

In total, this updated review includes 15 randomised clinical trials, published between 2002 and 2015, involving 71,422 participants (B-PROOF 2015; BVAIT 2009; CHAOS 2002; CSPPT 2015; FOLARDA 2004; GOES 2003; HOPE-2 2006; Li 2015a; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008). See Figure 1 for details.

Figure 1. Study flow diagram.

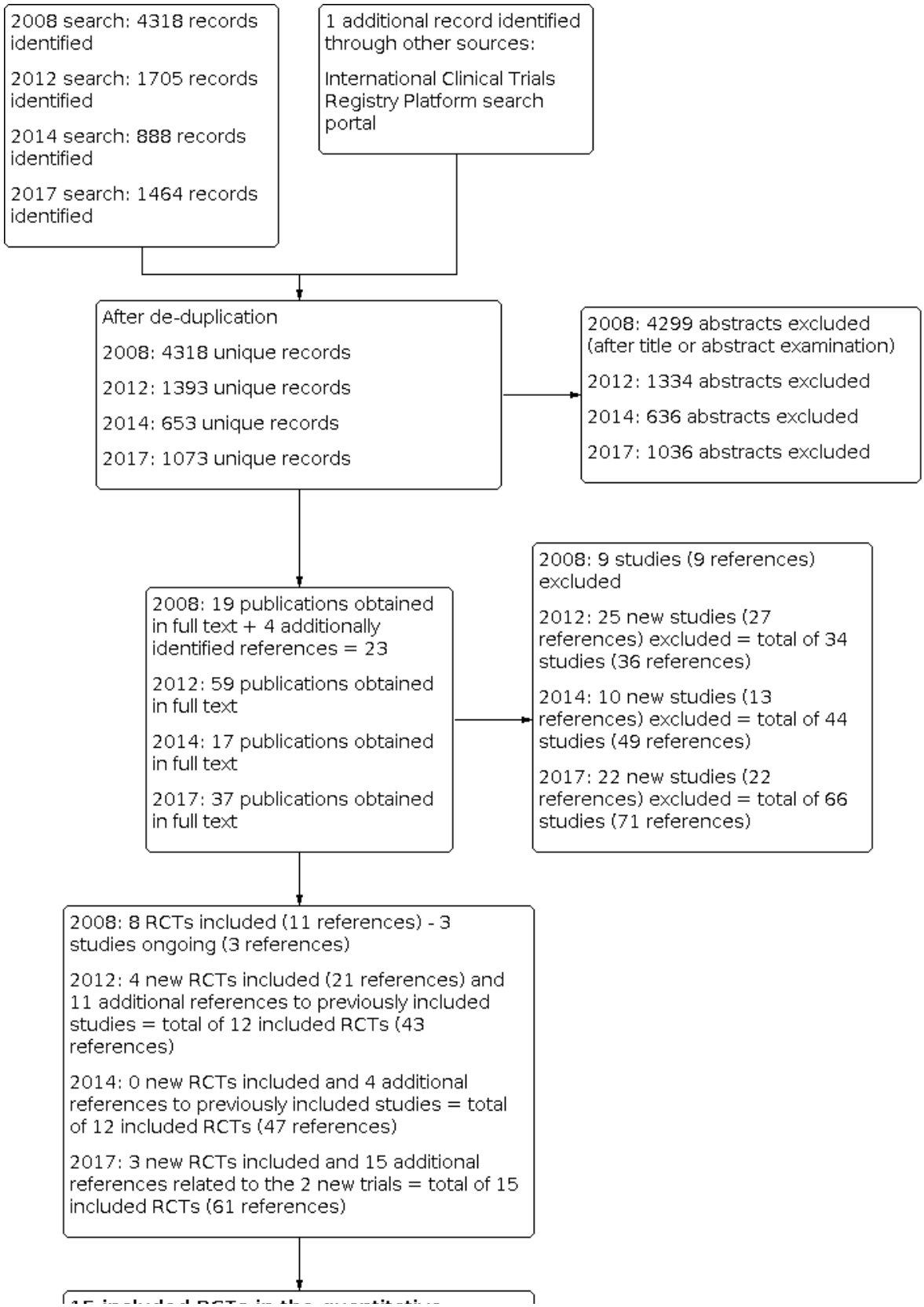
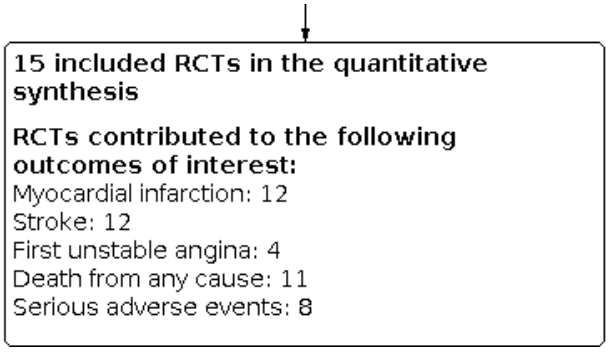


Figure 1. (Continued)



These trials are described in the section 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

Thirteen trials were conducted in participants with known cardiovascular disease, such as coronary artery disease, myocardial infarction, stable angina, unstable angina, stroke or intermittent claudication (B-PROOF 2015; BVAIT 2009; CHAOS 2002; FOLARDA 2004; GOES 2003; HOPE-2 2006; Li 2015a; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VITATOPS 2010; WAFACS 2008; WENBIT 2008), one trial included participants without any history of cardiovascular disease (CSPPT 2015); a further trial explicitly included participants with a history of non-disabling cerebral infarction (VISP 2004).

Fourteen trials included participants with at least one of the following known cardiovascular risk factors: diabetes mellitus, hypertension, elevated total cholesterol, current smoking, or low high-density lipoprotein (HDL) cholesterol (B-PROOF 2015; BVAIT 2009; CSPPT 2015; FOLARDA 2004; GOES 2003; HOPE-2 2006; Li 2015a; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008). This aspect was unclear for CHAOS 2002. One trial (WAFACS 2008) included participants with three or more coronary risk factors. One trial explicitly excluded participants with previously known hyperhomocysteinaemia (total plasma homocysteine > 18 µmol/L) (FOLARDA 2004).

BVAIT 2009 included participants with hyperhomocysteinaemia without diabetes and cardiovascular disease. HOPE-2 2006 included participants without a history of coronary heart disease (CHD). WAFACS 2008 only included female participants. Li 2015a included hypertensive females with hyperhomocysteinaemia.

Eleven trials included more than 1000 participants (B-PROOF 2015; CSPPT 2015; CHAOS 2002; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008). Two trials only included elderly participants (B-PROOF 2015; Li 2015a).

Ten trials were compared with placebo (B-PROOF 2015; 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 two trials were randomised controlled trials (Li 2015a; VISP 2004), which compared doses of homocysteine-lowering interventions. One trial compared antihypertensive medication plus a homocysteine-lowering intervention versus antihypertensive medication alone (CSPPT 2015).

The intervention assessed by most of the trials was a combination of vitamins B6, B9 and B12 (B-PROOF 2015; BVAIT 2009; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008). Five trials only included vitamin B9 as intervention (CSPPT 2015; CHAOS 2002; FOLARDA 2004; GOES 2003; Li 2015a). 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 medications. SU.FOL.OM3 2010 reported omega 3 polyunsaturated fatty acids used as concomitant medications. B-PROOF 2015 reported vitamin D3 use as a concomitant medication. Li 2015a reported restriction of salt intake and administration of vitamin B12 as a concomitant medication. CSPPT 2015 described the use of antihypertensive medications, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, calcium channel blockers, diuretics, β-Blockers, lipid-lowering medications, glucose-lowering medications and antiplatelet medications concomitantly.

Three trials were conducted in a "fortified" population (BVAIT 2009; VISP 2004; WAFACS 2008). The programme was described as a "...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 10 were carried out in non-fortified populations (B-PROOF 2015; CSPPT 2015; CHAOS 2002; FOLARDA 2004; GOES 2003; Li 2015a; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; WENBIT 2008).

Twelve trials used composite outcomes in their analyses (B-PROOF 2015; CSPPT 2015; 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). Fourteen trials had stroke as the endpoint (B-PROOF 2015; BVAIT 2009; CSPPT 2015; FOLARDA 2004; GOES 2003; HOPE-2 2006; Li 2015a; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008). Fourteen trials assessed the impact of the intervention on myocardial infarction rates (B-PROOF 2015;

BVAIT 2009; CHAOS 2002; CSPPT 2015; FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008). One trial included angina pectoris as a component of composite outcomes (B-PROOF 2015).

Thirteen studies reported the sample size calculation (B-PROOF 2015; BVAIT 2009; CSPPT 2015; 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 a 20% and 50% reduction in endpoints.

Concentrations of total homocysteine blood levels at baseline were reported in 13 trials (B-PROOF 2015; BVAIT 2009; CSPPT 2015; CHAOS 2002; GOES 2003; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008 WENBIT 2008). Six trials reported the total homocysteine blood levels at the end of follow-up (B-PROOF 2015; CHAOS 2002; HOPE-2 2006; NORVIT 2006; VISP 2004; WAFACS 2008). WENBIT 2008 described total homocysteine blood levels after one year of the intervention. CHAOS 2002 did not report total homocysteine blood levels at baseline or at the end of follow-up in the control arm. GOES 2003 reported total homocysteine blood levels at baseline and at the end follow-up, but only for the intervention arm and not for the control arm. FOLARDA 2004 and Li 2015a did not report the circulating total homocysteine blood levels in either group.

Definitions used for defining myocardial infarction, stroke, unstable angina and death (all-cause) are described in Appendix 6.

Excluded studies

This review has 66 references excluded (44 in the prior versions and 22 in this update), which are described in the table of Characteristics of excluded studies. These studies were mainly systematic reviews, RCTs with a follow-up of less of one year, and non-RCTs.

Risk of bias in included studies

The risk of bias in the included trials is summarised in Figure 2 and Figure 3, and detailed in the Characteristics of included studies tables. See Appendix 5 for details.

Figure 2. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

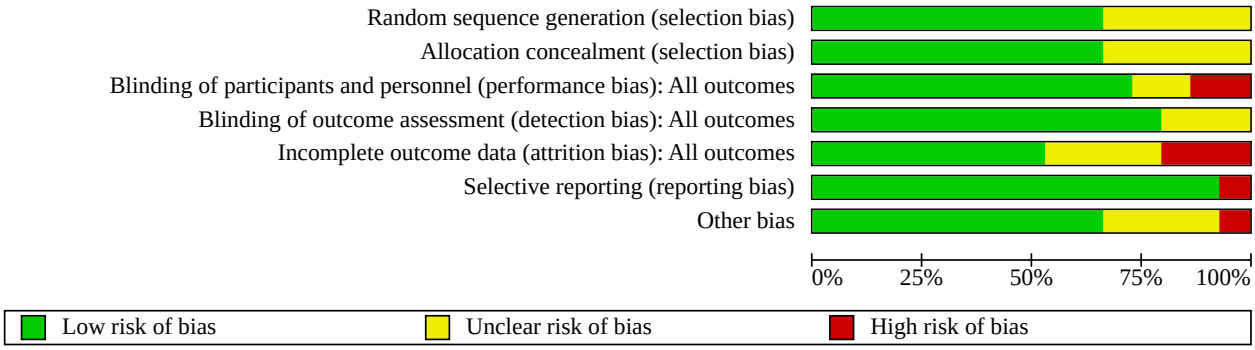
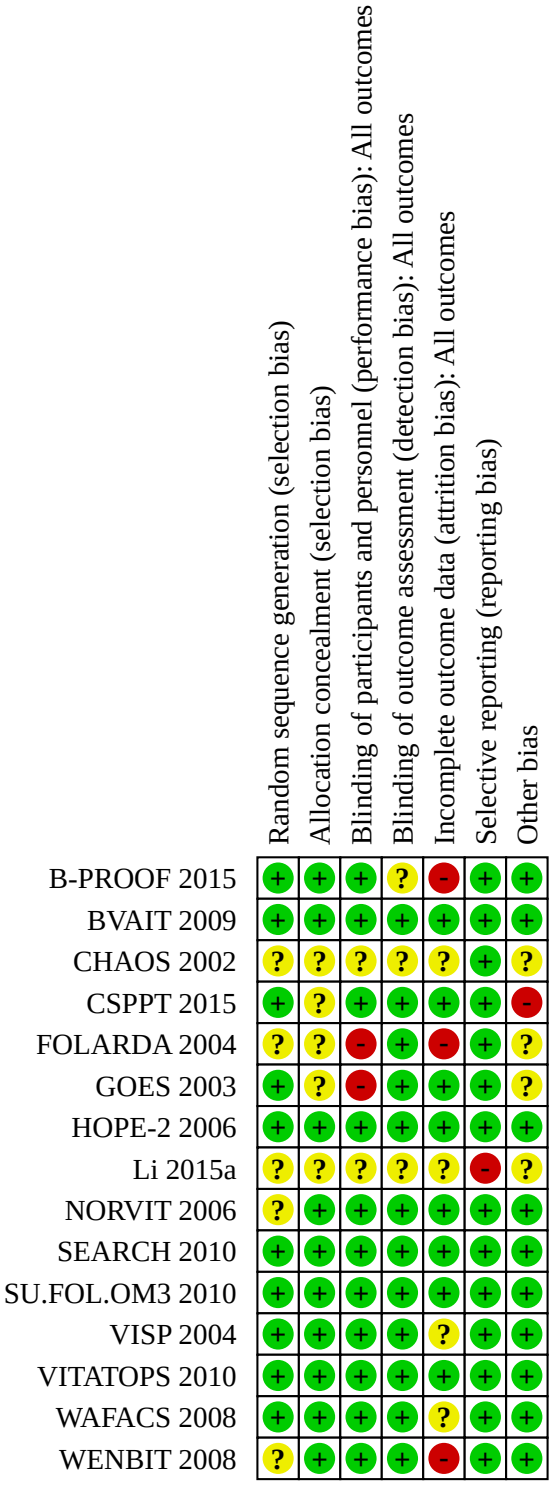


Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



Allocation

Random sequence generation

The risk of bias arising from the method of generation of the allocation sequence was low in 10 trials (B-PROOF 2015; BVAIT 2009; CSPPT 2015; GOES 2003; HOPE-2 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008). Five trials had an unclear risk for this domain (CHAOS 2002; FOLARDA 2004; Li 2015a; NORVIT 2006; WENBIT 2008).

Allocation concealment

We rated the risk of bias arising from the method of allocation concealment as low in 10 trials (B-PROOF 2015; BVAIT 2009; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008). Five trials showed an unclear risk for this domain (CHAOS 2002; CSPPT 2015; FOLARDA 2004; GOES 2003; Li 2015a).

Blinding

We rated the risk of bias arising from lack of blinding of participants and personnel as low in 11 trials (B-PROOF 2015; BVAIT 2009; CSPPT 2015; 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 two trials (CHAOS 2002; Li 2015a). We rated the risk of bias arising from lack of blinding as high in two trials (FOLARDA 2004; GOES 2003).

Blinding of outcome assessment (detection bias)

We rated the risk of bias arising from lack of blinding of outcome assessment as low in 12 trials (BVAIT 2009; CSPPT 2015; FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008). The risk of bias from unblinding was unclear in three trials (B-PROOF 2015; CHAOS 2002; Li 2015a).

Incomplete outcome data

We rated the risk of attrition bias as low in eight trials (BVAIT 2009; CSPPT 2015; 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 three trials (B-PROOF 2015; FOLARDA 2004; WENBIT 2008). We rated the risk of bias as unclear in four trials (CHAOS 2002; Li 2015a; VISP 2004; WAFACS 2008).

Selective reporting

Fourteen trials had a low risk of bias in this domain. One trial was rated as having high risk of bias for selective reporting (Li 2015a) due to lack of information on adverse events.

Other potential sources of bias

Ten trials had a low risk of bias due to other sources of bias not identified (B-PROOF 2015; BVAIT 2009; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008). Four trials had an unclear risk of bias (CHAOS 2002; FOLARDA 2004; GOES 2003; Li 2015a). One trial was rated as having high risk of bias (CSPPT 2015).

Overall risk of bias

Nine trials were rated as having low risk of bias (BVAIT 2009; CSPPT 2015; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008).

Effects of interventions

See: **Summary of findings 1** Homocysteine-lowering interventions (Vitamin B6 (pyridoxine; pyridoxal); B9 (folic acid) or B12 (cyanocobalamin) compared with placebo or standard care for preventing cardiovascular events; **Summary of findings 2** Homocysteine-lowering interventions (high dose) compared with homocysteine-lowering interventions (low dose) for preventing cardiovascular events; **Summary of findings 3** Enalapril plus folic acid compared with enalapril for adults with hypertension

The results are based on 71,422 participants in 15 randomised clinical trials (B-PROOF 2015; BVAIT 2009; CHAOS 2002; CSPPT 2015; FOLARDA 2004; GOES 2003; HOPE-2 2006; Li 2015a; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VISP 2004; VITATOPS 2010; WAFACS 2008; WENBIT 2008).

See Summary of findings 1; Summary of findings 2 and Summary of findings 3 for details.

Primary outcomes

Non-fatal or fatal myocardial infarction

Homocysteine-lowering interventions compared with placebo or conventional care

A meta-analysis of 12 randomised clinical trials (46,699 participants) showed uncertainty in the effect on non-fatal or fatal myocardial infarction between homocysteine-lowering interventions and placebo or conventional care (1788/25,051 (7.14%) versus 1290/21,648 (5.96%); risk ratio (RR) 1.02, 95% confidence interval (CI) 0.95 to 1.10; P value = 0.56, I^2 = 0%; high-quality evidence) (B-PROOF 2015; 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) (Analysis 1.1). The Bayes factor was 1.04, which means that evidence is insensitive, the data are equally well predicted by both models and the evidence does not favour either model over the other. Trial sequential analysis for myocardial infarction suggested that no more trials are needed to disprove a 10% relative risk reduction with the intervention. Smaller risk reductions might still require further trials (Figure 4). There was a low risk of publication bias (P value = 0.88, Harbord test; P value = 0.86, Peters test). Figure 5 and Figure 6 show funnel and contour-enhanced funnel plots, respectively.

Figure 4. Trial Sequential Analysis for homocysteine-lowering interventions versus placebo on myocardial infarction. The diversity-adjusted required information size (DARIS) was calculated based on an expected relative risk reduction (RRR) of 10% from proportion event in control (Pc) group of 5.95% with an alpha of 5% and beta of 20%. Cumulative Z-curve (blue line) reached futility area which means that no more trials are needed.

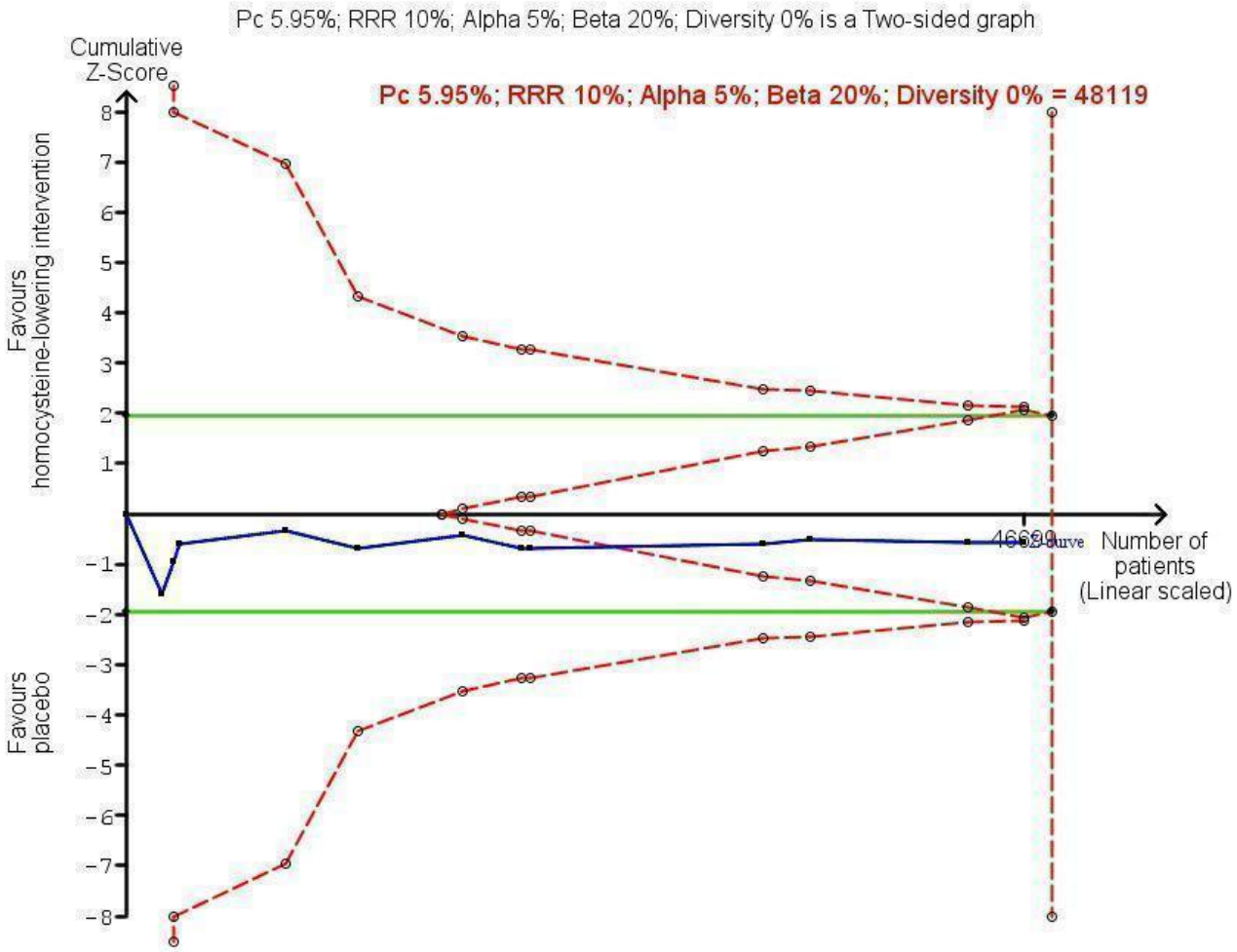


Figure 5. Funnel plot of comparison: 1 Homocysteine-lowering interventions versus placebo, outcome: 1.1 Myocardial infarction.

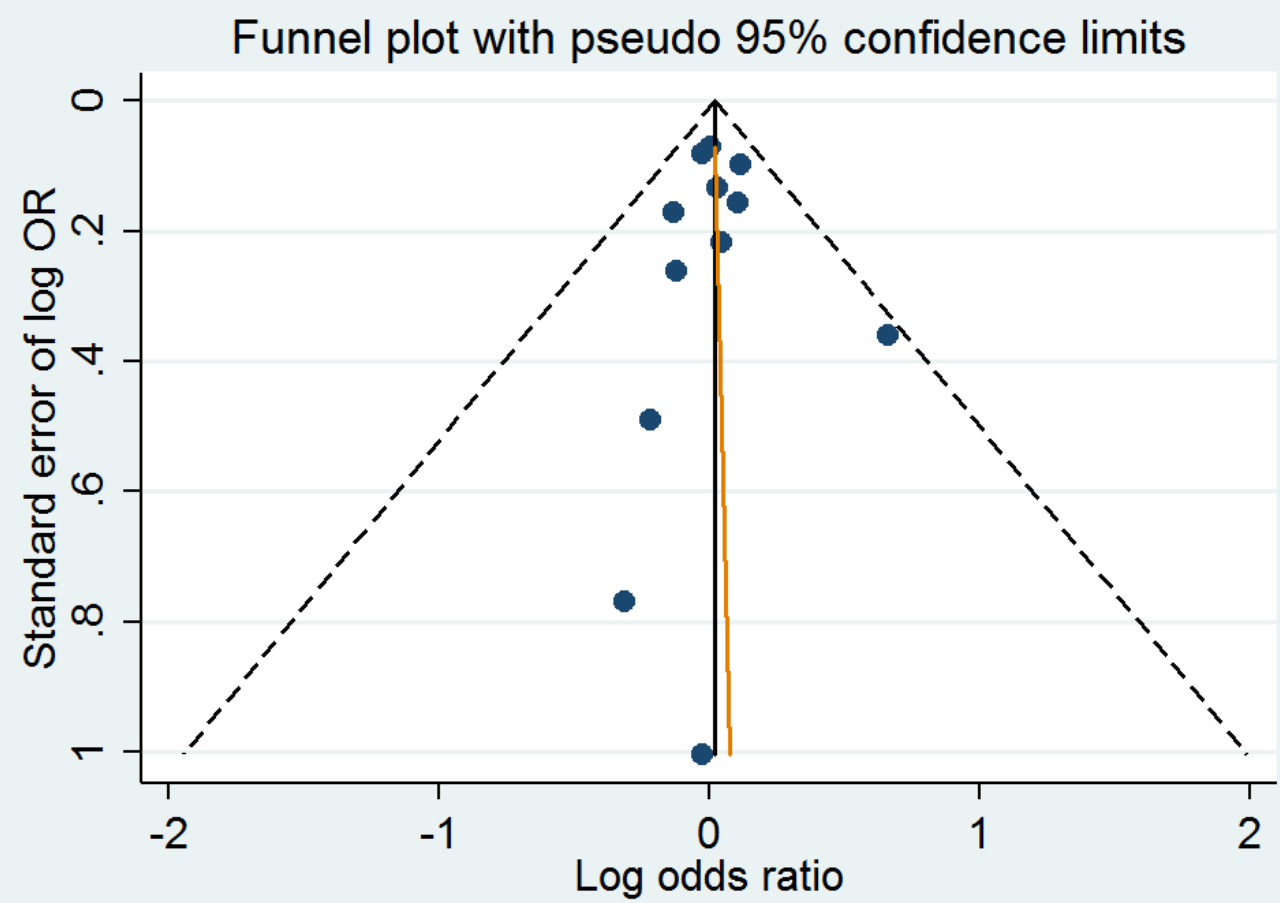
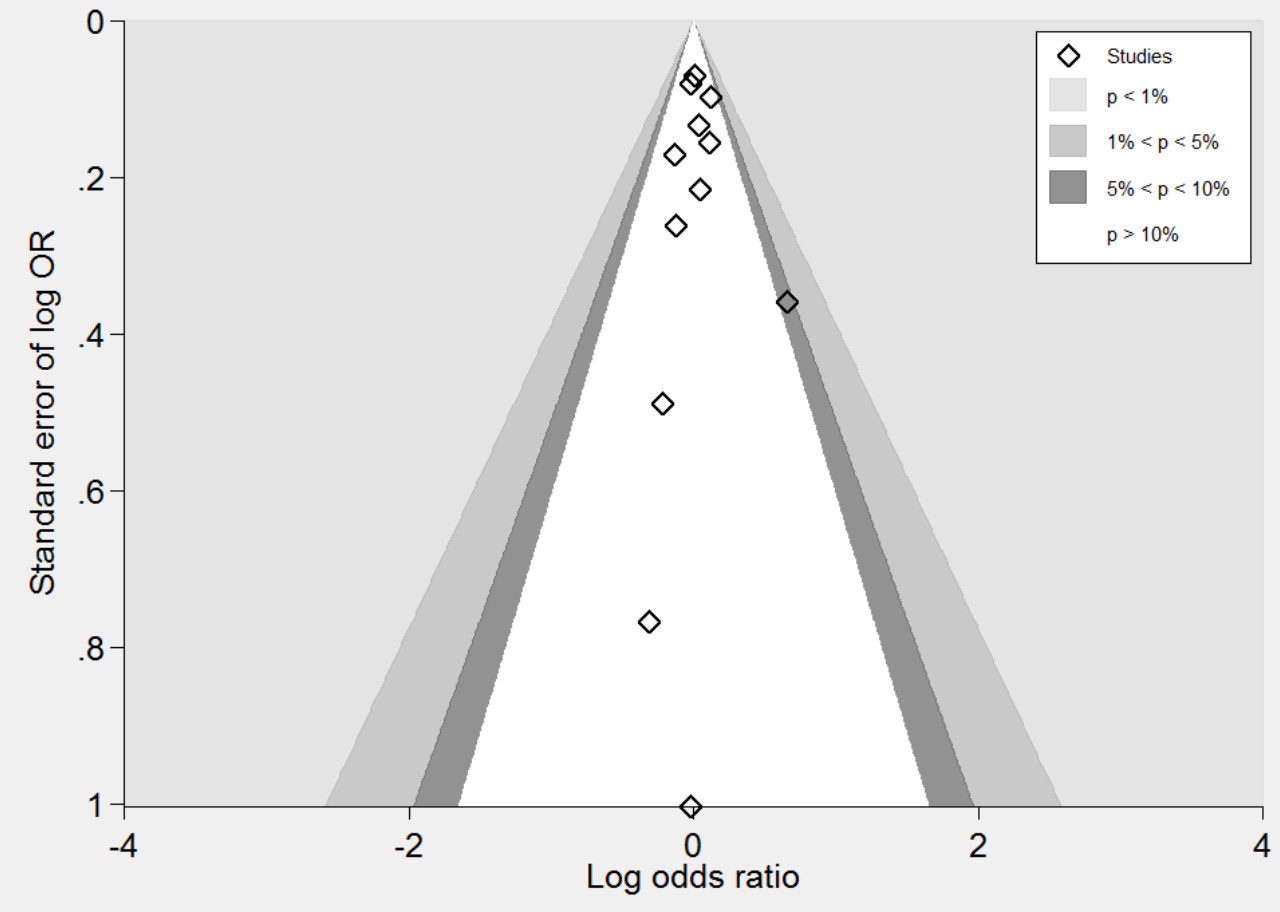


Figure 6. Contour-enhanced funnel plot of comparison: 1 Homocysteine-lowering interventions versus placebo, outcome: 1.1 Myocardial infarction.



Subgroup trials with a low risk of bias

A meta-analysis of six trials (37,442 participants) found uncertainty over the effect of intervention in non-fatal or fatal myocardial infarction rates (1517/19,649 (7.72%) versus 1161/17,793 (6.53%); RR 1.01, 95% CI 0.94 to 1.09, P value = 0.79, I^2 = 0%) (HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VITATOPS 2010; WAFACS 2008). (Analysis 2.1).

Subgroup analysis comparing trials including participants without or with history of cardiovascular disease

One trial (490 participants) including participants without cardiovascular disease found uncertainty between intervention and placebo groups regarding non-fatal or fatal myocardial infarction (2/248 (0.81%) versus 2/242 (0.83%); RR 0.98, 95% 0.14 to 6.87, P value = 0.98) (BVAIT 2009). A meta-analysis of 11 trials (46,209 participants) including participants with a history of cardiovascular disease showed that there was no difference in non-fatal or fatal myocardial infarction between intervention and placebo groups (1786/24,803 (7.20%) versus 1288/21,406 (6.02%); RR 1.02, 95% CI 0.95 to 1.10, I^2 = 9%) (B-PROOF 2015; FOLARDA 2004; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VITATOPS 2010; WAFACS 2008; WENBIT 2008). Testing for subgroup differences found no significant difference (P value = 0.96 and I^2 = 0%). Analysis 3.1.

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

One trial (3649 participants) found a lower proportion had non-fatal or fatal myocardial infarctions in participants assigned to a high dose of homocysteine-lowering interventions compared with those receiving a low dose of homocysteine-lowering interventions (72/1814 (3.97%) versus 81/1835 (4.41%); RR 0.90, 95% CI 0.66 to 1.23, P value = 0.50; moderate-quality evidence) (VISP 2004) (Analysis 1.1). The Bayes factor was 1.06, which means that evidence is insensitive, the data are equally well predicted by both models and the evidence does not favour either model over the other.

Homocysteine-lowering treatment (folic acid) plus antihypertensive therapy (enalapril) versus antihypertensive therapy (enalapril)

One trial (20,702 participants) found uncertainty in the rates of non-fatal or fatal myocardial infarction between intervention and control groups (25/10,348) (0.24%) versus 24/10,354 (0.23%); RR 1.04, 95% CI 0.60 to 1.82, P value = 0.88; moderate-quality evidence) (Analysis 1.1). The Bayes factor was 0.97 which means that evidence is insensitive, the data are equally well predicted by both models and the evidence does not favour either model over the other.

Subgroup analysis for missing data

One trial (20,635 participants) comparing the combination of folic acid plus enalapril with enalapril alone showed inconsistent results in terms of non-fatal or fatal myocardial infarction, according to per protocol analysis (25/10,316 (0.24%) versus 24/10,319 (0.23%); RR 1.04, 95% CI 0.60 to 1.82, P value = 0.89), best-worst case scenario (25/10,348 (0.24%) versus 59/10,354 (0.57%); RR 0.42, 95% CI 0.27 to 0.68, P value = 0.0003) and worst-best case scenario (57/10,348 (0.55%) versus 24/10,354 (0.23%); RR 2.38, 95% CI 1.48 to 3.83, P value = 0.0004). Testing for subgroup differences found a significant difference (P value <0.0001 and I^2 = 92%) (CSPPT 2015). Analysis 4.1.

Non-fatal or fatal stroke

Homocysteine-lowering interventions compared with placebo

A meta-analysis of ten trials (44,224 participants) showed a risk reduction in non-fatal or fatal stroke in participants assigned to homocysteine-lowering interventions compared with placebo (1014/23,809 (4.26%) versus 1034/20,415 (5.06%); RR 0.90, 95% CI 0.82 to 0.99, P value = 0.03, I^2 = 8%, high-quality evidence) (B-PROOF 2015; BVAIT 2009; FOLARDA 2004; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VITATOPS 2010; WAFACS 2008; WENBIT 2008) (Analysis 1.2). The Bayes factor was 5.84 which means that it is 5.84 times more likely that homocysteine-lowering interventions reduce non-fatal or fatal stroke compared with placebo. Trial sequential analysis for stroke suggested that no more trials are needed to disprove a 10% relative risk reduction with intervention. Smaller risk reductions might still require further trials (Figure 7). There was a low risk of publication bias (P value = 0.368, Harbord test; P value = 0.393, Peters test). Figure 8 and Figure 9 show funnel and contour-enhanced funnel plots, respectively.

Figure 7. Trial Sequential Analysis for homocysteine-lowering interventions versus placebo on stroke. The diversity-adjusted required information size (DARIS) was calculated based on an expected relative risk reduction (RRR) of 10% from proportion event in control (Pc) group of 5% with an alpha of 5% and beta of 20%.

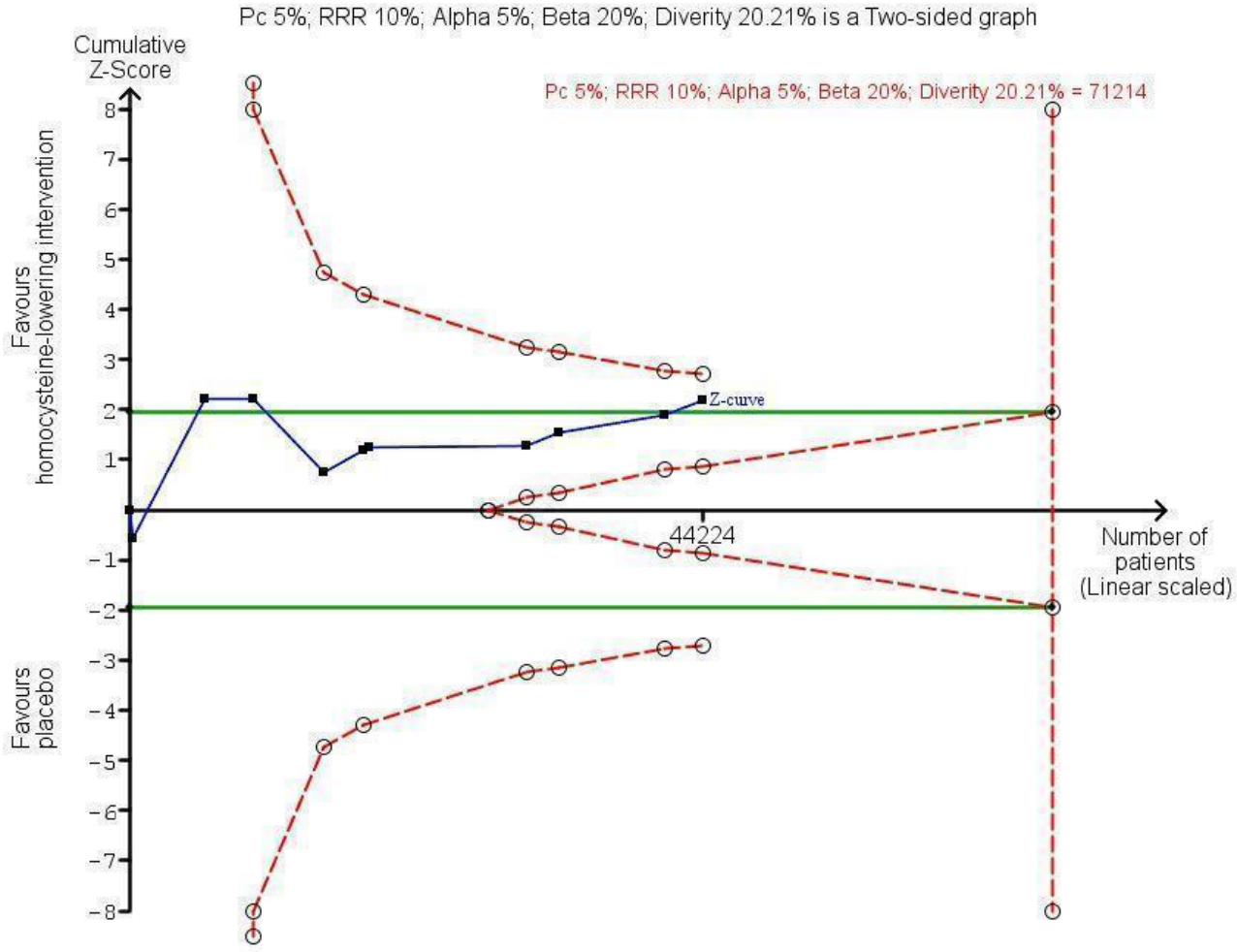


Figure 8. Funnel plot of comparison: 1 Homocysteine-lowering interventions versus placebo, outcome: 1.2 Stroke.

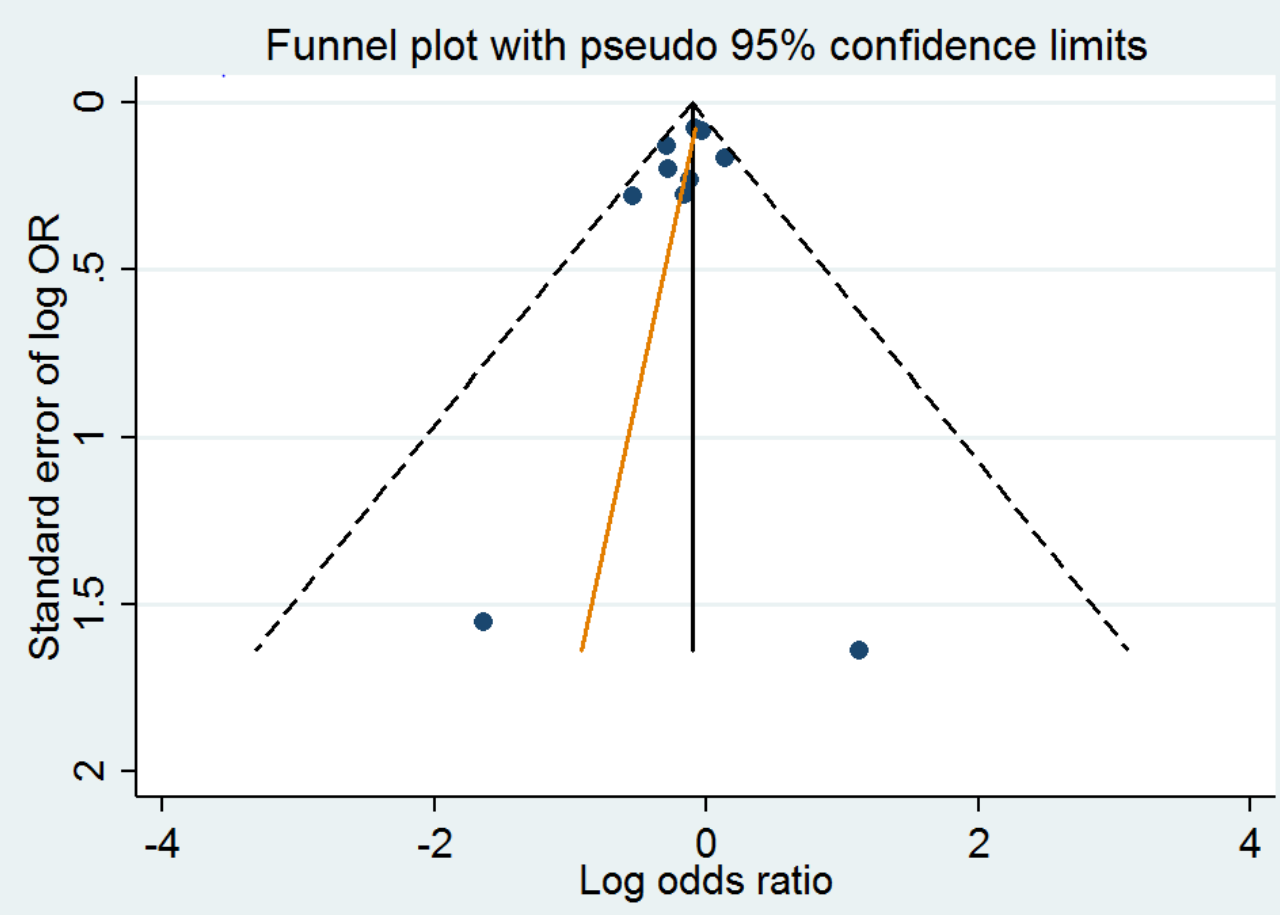
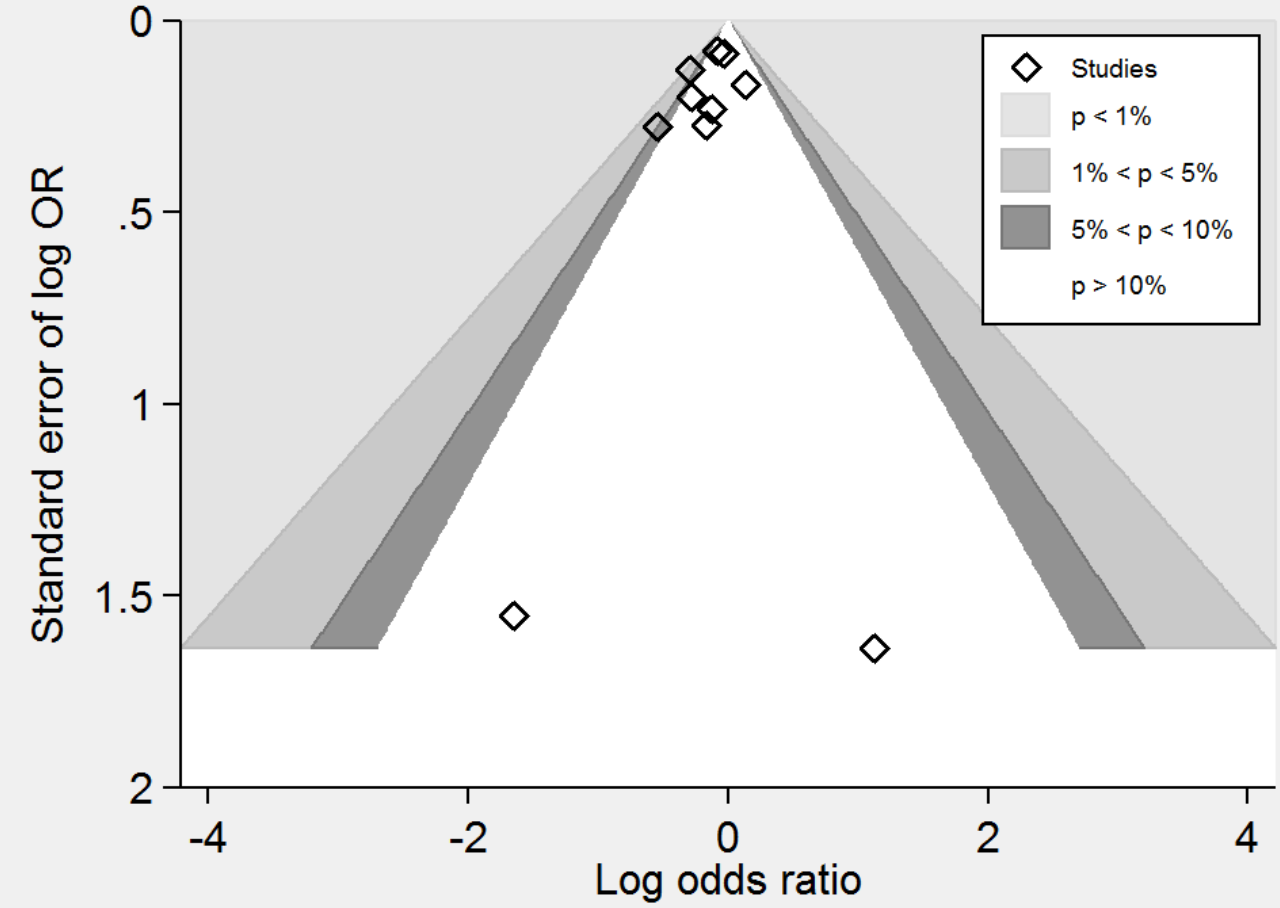


Figure 9. Contour-enhanced funnel plot of comparison: 1 Homocysteine-lowering interventions versus placebo, outcome: 1.2 Stroke.



Subgroup trials with a low risk of bias

A meta-analysis of six trials (37,442 participants) found uncertainty in differences between non-fatal or fatal stroke rates between intervention and placebo groups (919/19,649 (4.68%) versus 953/17,793 (5.36%); RR 0.90, 95% CI 0.80 to 1.02, P value = 0.10, I^2 = 32%) (HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VITATOPS 2010; WAFACS 2008). (Analysis 2.2).

Subgroup analysis comparing trials including participants without or with history of cardiovascular disease

One trial (490 participants) including participants without cardiovascular disease found uncertainty between intervention and placebo groups regarding the rates of non-fatal or fatal stroke (0/248 (0%) versus 2/242 (0.83%); RR 0.20, 95% 0.01 to 4.04, P value = 0.29) (BVAIT 2009). A meta-analysis of nine trials (43,734 participants) including participants with history of cardiovascular disease showed evidence of effect favouring intervention group versus placebo group in terms of non-fatal or fatal stroke rates (1014/23,561 (4.30%) versus 1032/20,173 (5.12%); RR 0.90, 95% CI 0.82 to 0.99, I^2 = 9%) (B-PROOF 2015; FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VITATOPS 2010; WAFACS 2008; WENBIT 2008). Testing for subgroup differences found no significant difference (P 0.32 and I^2 = 0%). Analysis 3.2.

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

A meta-analysis of two trials (3929 participants) showed uncertainty between the effects of high dose versus low dose of homocysteine-lowering interventions with regards to non-fatal or fatal stroke (211/1958 (10.78%) versus 221/1971 (11.21%); RR 0.90, 95% CI 0.66 to 1.22; I^2 = 72%; very low-quality evidence) (Li 2015a; VISP 2004) (Analysis 1.2). The Bayes factor was 1.06, which means that evidence is insensitive, the data are equally well predicted by both models and the evidence does not favour either model over the other.

We detected high statistical heterogeneity, as conveyed by the I^2 value (72%), and therefore we further explored by type of planned intervention.

One trial (3649 participants) comparing a combination of homocysteine-lowering interventions (folic acid, vitamin B6 and vitamin B12), either at high dose (2.5 mg folic acid; 0.4 mg vitamin B12; 25 mg vitamin B6), or low dose (20 micrograms folic acid; 6 micrograms vitamin B12; 200 micrograms vitamin B6) found uncertainty over the effects on non-fatal or fatal stroke rates (152/1814 (8.38%) versus 148/1835 (8.07%); RR 1.04, 95% CI 0.84 to 1.29; P value = 0.73) (VISP 2004). Analysis 5.1

One trial (280 participants) conducted only with elderly female participants, compared folic acid at high dose (0.8 mg) plus vitamin B12 (500 µg) versus folic acid at low dose (0.4 mg) plus vitamin B12 (500 µg). It found a lower proportion of non-fatal or fatal strokes in participants assigned to high-dose folic acid than those receiving a low-dose folic acid (59/144 (40.97%) versus 73/136 (53.68%); RR 0.76, 95% CI 0.59 to 0.98; P value = 0.03) (Li 2015a). Analysis 5.1

Homocysteine-lowering treatment (folic acid) plus antihypertensive therapy (enalapril) versus antihypertensive therapy (enalapril)

One trial (20,702 participants) found a reduced risk of non-fatal or fatal stroke in participants receiving enalapril plus folic acid compared with participants receiving enalapril as monotherapy (281/10348 (2.72%) versus 354/10354 (3.42%); RR 0.79, 95% CI 0.68 to 0.93, P value = 0.003; NNTB 143, 95% CI 85 to 428, high-quality evidence) (Analysis 1.2). The Bayes factor was 31.9 which means that it is 31.9 times more likely that homocysteine-lowering treatment (folic acid) plus antihypertensive therapy (enalapril) versus antihypertensive therapy (enalapril) alone reduces non-fatal or fatal stroke. The fragility Index was 23.

Subgroup analysis for missing data

The overall incidence of non-fatal or fatal stroke seemed to be reduced in people assigned to a combination of folic acid plus enalapril versus those allocated to enalapril alone: per protocol analysis (281/10,316 (2.72%) versus 354/10,319 (3.43%); RR 0.79, 95% CI 0.68 to 0.93; P value = 0.003), best-worst case scenario (281/10,348 (2.72%) versus 389/10,354 (3.76%); RR 0.72, 95% CI 0.62 to 0.84; P value = 0.0001) and worst-best case scenario (313/10,348 (3.02%) versus 354/10,354 (3.42%); RR 0.88, 95% CI 0.76 to 1.03; P value = 0.11). Test for subgroup differences: P = 0.18, I^2 = 42.5%. (CSPPT 2015). Analysis 4.2.

Secondary outcomes

First unstable angina pectoris episode requiring hospitalisation

Homocysteine-lowering interventions compared with placebo

A meta-analysis of four trials (12,644 participants) showed uncertainty between the effects intervention compared with placebo on the rate of unstable angina requiring hospitalisation (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 an uncertain effect in the hospitalisation for heart failure rates between intervention and placebo groups (202/2758 (7.32%) versus 174/2764 (6.30%); 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

A meta-analysis of 11 trials (44,817 participants) found uncertainty between the effects of intervention versus placebo on the rates of death from any cause (2821/24,109 (11.70%) versus 2544/20,708 (12.29%); RR 1.01, 95% CI 0.96 to 1.06, P value = 0.68, I^2 = 0%, high-quality evidence) (B-PROOF 2015; 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). The Bayes factor was 1.05, which means that evidence is insensitive, the data are equally well predicted by both models and the evidence does not favour either model over the other. Trial sequential analysis for stroke suggested that no more trials are needed to disprove a 10% relative risk reduction with intervention. Smaller risk reductions might still require further trials (Figure 10). There was a low risk of publication bias (P value = 0.95, Harbord test; P value = 0.82, Peters test). Figure 11 and Figure 12 show funnel and contour-enhanced funnel plots, respectively.

Figure 10. Trial Sequential Analysis for homocysteine-lowering interventions versus placebo on death from any cause. The diversity-adjusted required information size (DARIS) was calculated based on an expected relative risk reduction (RRR) of 12% from proportion event in control (Pc) group of 11.7% with an alpha of 5% and beta of 20%. Cumulative Z-curve (blue line) reached futility area which means that no more trials are needed.

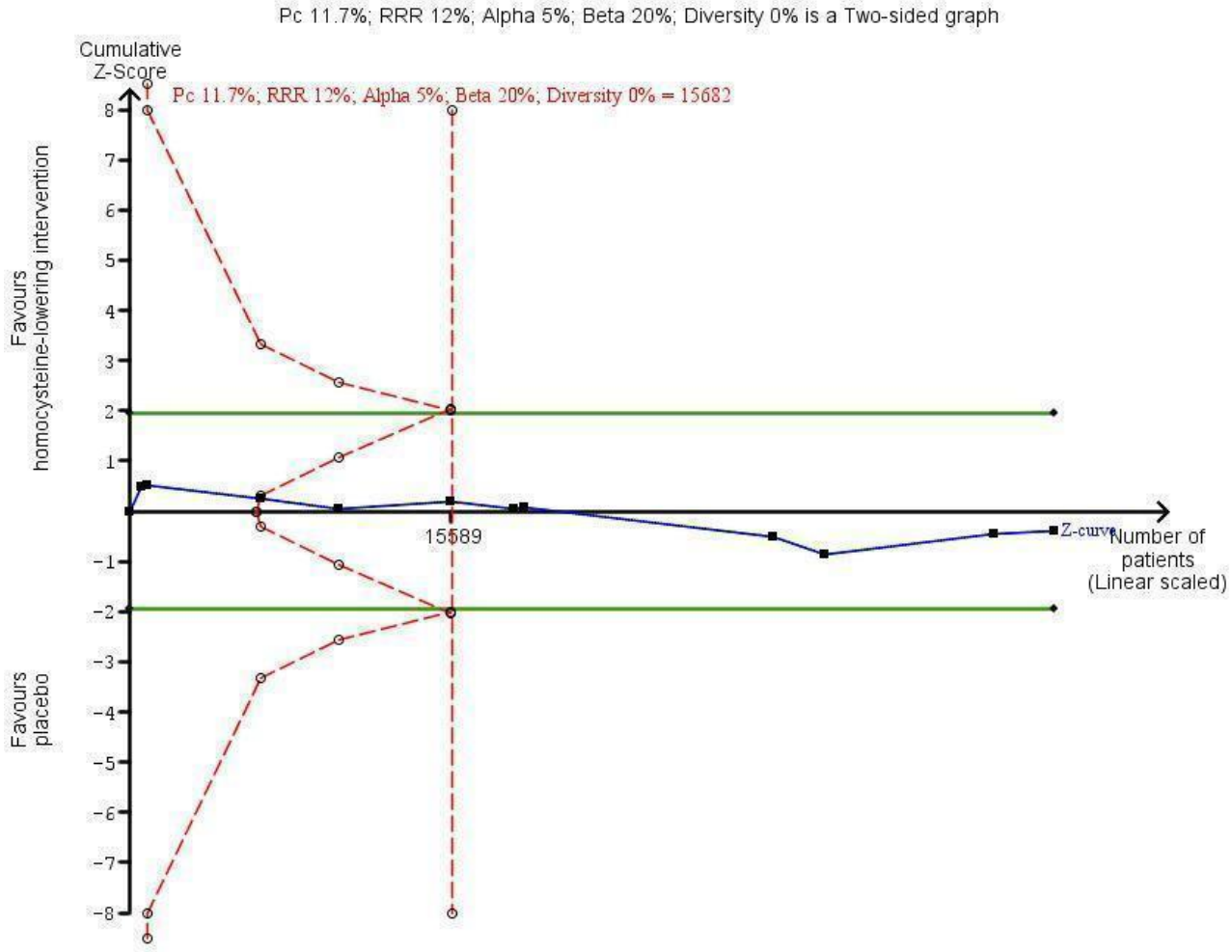


Figure 11. Funnel plot of comparison: 1 Homocysteine-lowering interventions versus placebo, outcome: 1.4 Death from any cause.

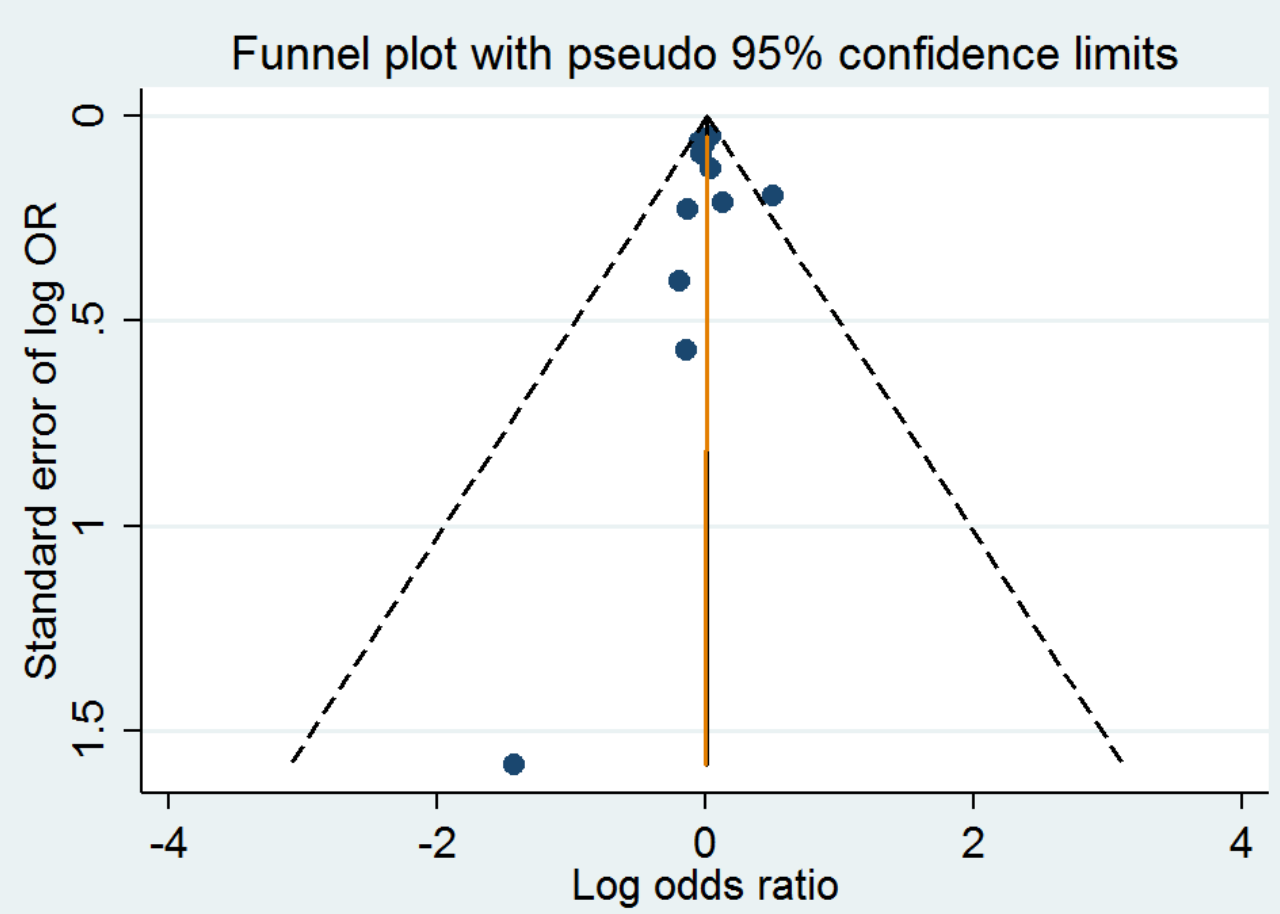
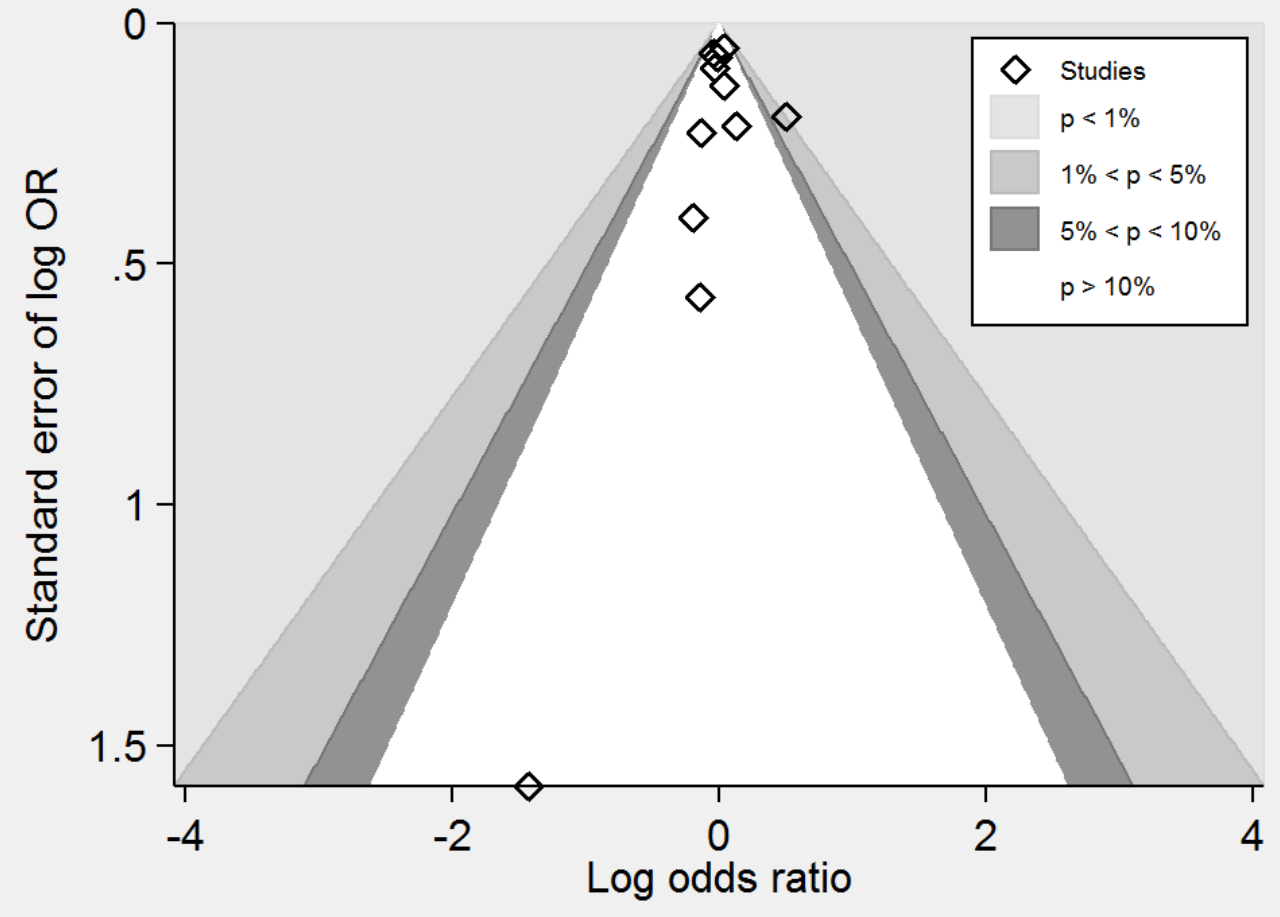


Figure 12. Contour-enhanced funnel plot of comparison: 1 Homocysteine-lowering interventions versus placebo, outcome: 1.4 Death from any cause.



Subgroup trials with a low risk of bias

A meta-analysis of seven trials (37,932 participants) found uncertainty between the effects of intervention versus placebo in rates of death from any cause (2145/19,897 (10.78%) versus 1923/18,035 (10.66%); RR 1.03, 95% CI 0.95 to 1.12; P value = 0.48; I^2 = 41%) (BVAIT 2009; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VITATOPS 2010; WAFACS 2008). (Analysis 2.3).

Subgroup analysis comparing trials including participants without or with history of cardiovascular disease

One trial (490 participants) including participants without cardiovascular disease found uncertainty between the effects of intervention versus placebo in rates of death from any cause (0/248 (0%) versus 2/242 (0.83%); RR 0.20, 95% 0.01 to 4.04, P value = 0.29) (BVAIT 2009). A meta-analysis of 10 trials (44,327 participants) including participants with history of cardiovascular disease showed conclusive evidence that there was no difference in rates of death from any cause between intervention and placebo groups (2821/23,861 (11.82%) versus 2542/20,466 (12.42%); RR 1.01, 95% CI 0.96 to 1.06, I^2 = 0%) (B-PROOF 2015; FOLARDA 2004; GOES 2003; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; VITATOPS 2010; WAFACS 2008; WENBIT 2008). Testing for subgroup differences found no significant difference (P 0.32 and I^2 = 0%). Analysis 3.3.

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

One trial (3649 participants) found uncertainty in mortality from any cause between intervention and control groups (99/1814 (5.46%) versus 117/1835 (6.38%); RR 0.86; 95% CI 0.66 to 1.11; P value = 0.24; moderate-quality evidence) (VISP 2004) (Analysis 1.4). The Bayes factor was 1.07 which means that evidence is insensitive, the data are equally well predicted by both models and the evidence does not favour either model over the other.

Homocysteine-lowering treatment (folic acid) plus antihypertensive therapy (enalapril) versus antihypertensive therapy (enalapril)

One trial (20,702 participants) found uncertainty between the effects of enalapril plus folic acid compared with participants receiving enalapril as monotherapy on mortality from any cause (302/10,348 (2.92%) versus 320/10,354 (3.09%); RR 0.94, 95% CI 0.81 to 1.10; P value = 0.47; high-quality evidence) (Analysis 1.4). The Bayes factor was 1.08 which means that evidence is insensitive, the data are equally well predicted by both models and the evidence does not favour either model over the other.

The combination of folic acid and enalapril seemed not to affect the incidence of death from any cause compared with enalapril alone. Per protocol analysis (302/10,316 (2.93%) versus 320/10,319

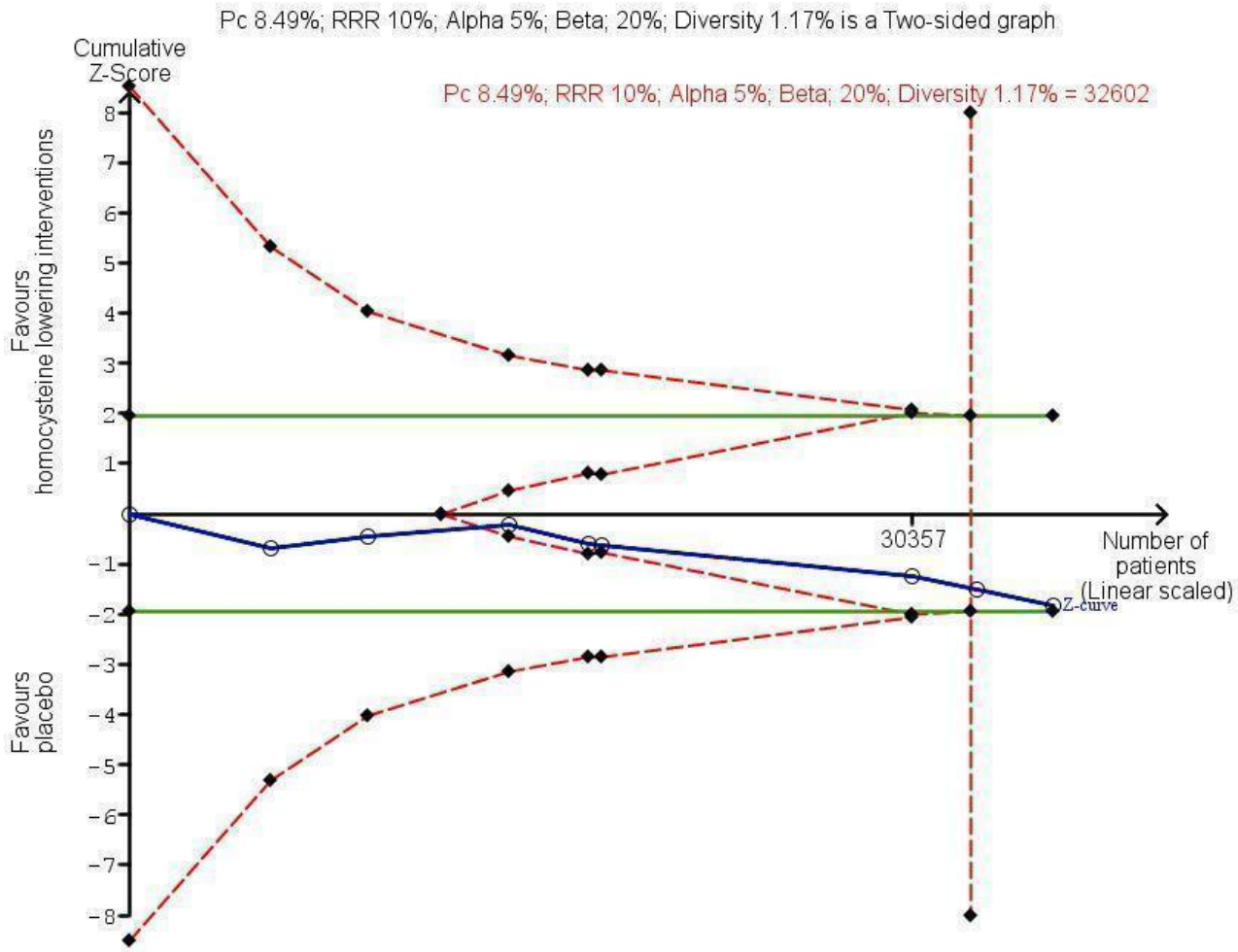
(3.10%); RR 0.94, 95% CI 0.81 to 1.10; P value = 0.47), best-worst case scenario (302/10,348 (2.92%) versus 355/10,354 (3.43%); RR 0.85, 95% CI 0.73 to 0.99; P value = 0.04) and (334/10,348 (3.23%) versus 320/10,354 (3.09%); RR 1.04, 95% CI 0.90 to 1.21; P value = 0.57). Test for subgroup differences: P = 0.17, I^2 = 43.3%. (CSPPT 2015). Analysis 4.3.

Serious or non-serious adverse events

Homocysteine-lowering interventions compared with placebo

A meta-analysis of eight trials (35,788 participants) assessing cancer incidence found uncertainty in the incidence of cancer in intervention and placebo groups (1621/19,591 (8.27%) versus 1376/16,197 (8.50%); RR 1.07, 95% CI 1.00 to 1.14, P value = 0.07, I^2 = 0%, high-quality evidence) (B-PROOF 2015; BVAIT 2009; HOPE-2 2006; NORVIT 2006; SEARCH 2010; SU.FOL.OM3 2010; WAFACS 2008; WENBIT 2008) (Analysis 1.5). Trial sequential analysis for adverse events suggested that no more trials are needed to disprove a 10% relative risk reduction (Figure 13).

Figure 13. Trial Sequential Analysis for homocysteine-lowering interventions versus placebo on adverse events (cancer). The diversity-adjusted required information size (DARIS) was calculated based on an expected relative risk reduction (RRR) of 10% from proportion event in control (Pc) group of 8.49% with an alpha of 5% and beta of 20%. Cumulative Z-curve (blue line) reached futility area which means that no more trials are needed.



A meta-analysis of three trials (13,802 participants) found uncertainty in terms of serious or non-serious adverse events rather than cancer between intervention and placebo groups (322/6903 (4.66%) versus 312/6899 (4.52%); RR 1.02, 95% 0.88 to 1.19; P value = 0.77, I^2 = 0%, high-quality evidence) (BVAIT 2009; SEARCH 2010; SU.FOL.OM3 2010). Analysis 1.6.

Homocysteine-lowering treatment (folic acid) plus antihypertensive therapy (enalapril) versus antihypertensive therapy (enalapril)

One trial (20,243 participants) found uncertainty in terms of cancer incidence between participants receiving enalapril plus folic acid compared with participants receiving enalapril as monotherapy (79/10,119 (0.78%) versus 82/10,124 (0.81%); RR 0.96, 95% CI 0.71 to 1.31; P value = 0.81; moderate-quality evidence) (Analysis 1.5).

DISCUSSION

Summary of main results

This updated Cochrane review of homocysteine-lowering interventions (B vitamins) for preventing cardiovascular events identified 15 randomised controlled trials incorporating 71,422 participants. Trials reported different combinations of homocysteine-lowering interventions compared with different control interventions (placebo: B-PROOF 2015; 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, homocysteine-lowering interventions at low dose: Li 2015a; VISP 2004, and antihypertensive medication (enalapril): (CSPPT 2015)). Overall, the trials had a low risk of bias and were adequately powered. Participants differed somewhat in cardiovascular risk levels (some with established cardiovascular disease (CVD), others at high risk of CVD), baseline total homocysteine blood levels, access to foods fortified with folic acid or not, different dosages of vitamins and different control groups, with treatment periods varying from two to seven years.

1. This review found no reduction of the incidence of either myocardial infarction (fatal or non-fatal) and death from any cause or an increasing risk of adverse events (cancer).

2. With regard to stroke (fatal or non-fatal), a meta-analysis of homocysteine-lowering interventions compared with placebo, and one mega-trial comparing folic acid plus enalapril with enalapril alone found a reduction of risk stroke in those treated. A meta-analysis of two trials comparing high dose versus low dose of homocysteine-lowering interventions did not find a difference in the rates of fatal or non-fatal strokes.

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 myocardial infarction (fatal or non-fatal) or death from any cause. We conducted a sensitivity analysis restricted to trials with low risk of bias for myocardial infarction and death from any cause. These results show consistency and are based on data from trials that included a broad range of participants 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 showed that the included trials may represent a broad picture of participants with a high risk of cardiovascular events.

With reference to stroke (non-fatal or fatal stroke), this update found that homocysteine-lowering interventions reduce the incidence of stroke compared with placebo or enalapril alone (Analysis 1.2). However, this result should be viewed with caution. In the trial sequential analysis graph called '*Trial sequential analysis on stroke in 10 trials investigating homocysteine-lowering interventions versus placebo*' (Figure 7), it was observed that after 44,224 participants the Z curve crossed the upper conventional alpha of 5%, but also the cumulative Z-curve crossed no the trial sequential alpha-spending monitoring boundaries also called as monitoring efficacy boundary (Roshanov 2017). This positive result appears to be weak, due to the 95% confidence interval reduction of risk of any stroke ranging between 1% and 18% and the very low basal risk. As is shown into Summary of findings 1, in the control group 51 people out of 1000 had a stroke (non-fatal or fatal) over 1 to 7.3 years, compared with 46 (95% CI 42 to 50) out of 1000 for the active treatment group.

CSPPT 2015 also found a positive result of enalapril plus folic acid compared with enalapril on incidence of any stroke. The trial had a duration of follow-up of five years. The absolute risk reduction in this trial was very low (0.7%), which explains the high number needed to treat for an additional beneficial outcome in this trial of 143 (95% CI 85 to 428). During five years between 85 and 428 hypertensive participants would need to take enalapril plus folic acid for to prevent one stroke. According to the Summary of findings 3, in the control group 34 people out of 1000 had a stroke (non-fatal or fatal) over five years, compared to 27 (95% CI 23 to 32) out of 1000 in the active treatment group. Therefore, the clinical difference is small between the groups, which is reflected in the sensitivity analysis shown into Analysis 4.2. We estimated the fragility index of CSPPT 2015 as 23, which denotes that if 23 patients in the experimental group were converted from not having the primary endpoint to having the primary endpoint, the study would lose statistical significance ($P > 0.05$). Furthermore, it is unknown whether such treatment would benefit a non-Chinese population. In both cases (meta-analysis with more than 40,000 and a trial with 20,000 participants) shows a highly significant statistical result, but it "may not represent a clinically important effect when treating patients in our daily lives." (Fuster 2015).

In conclusion, this updated version showed the same findings as the previous version (Martí-Carvajal 2015). It showed that supplementary vitamin B6, B12 and folic acid administration did not prevent cardiovascular events in participants with or without pre-existing cardiovascular disease. The trial sequential analysis for the same outcomes suggested that no further randomised trials were needed to assess the benefits and harms of homocysteine-lowering interventions to preventing cardiovascular events (Figure 4; Figure 7; Figure 10). Martí-Carvajal 2013and Martí-Carvajal 2015 found no effect of vitamin B-complex supplementation on rates of cancer (Figure 13). Bayes factors give prominence to these findings. There is a likelihood for reducing the stroke rate.

Quality of the evidence

We conducted GRADE assessments on outcomes using the meta-analysed trials.

Summary of findings 1 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.

Summary of findings 2 shows the quality of evidence for homocysteine-lowering interventions (high dose) compared with homocysteine-lowering interventions (low dose) for preventing cardiovascular events. The evidence was rated as moderate or very low due to imprecision i.e. low number of events, for risk of bias as one trial (Li 2015a) for having unclear risk of selection, conduction and detection biases, and for inconsistency ($I^2 = 72\%$).

Summary of findings 3 shows the quality of evidence for enalapril plus folic acid compared with enalapril for adults with hypertension. The evidence for stroke was rated as high.

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 2; Figure 3). This review might have a limitation due to paucity of data in terms of trials comparing 'head-to-head' homocysteine-lowering interventions. A strength of this new updated Cochrane review is to have shown an absence of asymmetry in almost all funnel plots and to discard publication bias using appropriate statistical methodology i.e. Harbord and Peters tests.

Agreements and disagreements with other studies or reviews

Our results are similar to other non-Cochrane reviews (Clarke 2010; Huang 2012; Huo 2012; Ji 2013). These four reviews differed in their eligibility criteria, i) resulting in 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 participants with kidney or renal disease, which is beyond 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 included five additional trials not considered in Clarke 2010, with 12,031 more participants, 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). Lai 2015b and colleagues reported any affect on the risk of cardiovascular disease such as suggested this Cochrane review.

Two randomised controlled trials (Jamison 2007; Vianna 2007), and two systematic reviews, (Jardine 2012; Pan 2012) involving participants with end-stage renal disease, found no effect of homocysteine-lowering interventions in preventing cardiovascular events.

Regarding cancer, this Cochrane review showed 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

In this third update of the review, there were no differences in effects of homocysteine-lowering interventions in the form of supplements of vitamins B6, B9 or B12 given alone or in combination comparing with placebo on myocardial infarction, death from any cause or adverse events. In terms of stroke, this review found a small difference in effect favouring homocysteine-lowering interventions in the form of supplements of vitamins B6, B9 or B12 given alone or in combination compared with placebo. There were uncertain effects of folic acid compared with enalapril plus folic acid on stroke; approximately 143 (95% CI 85 to 428) people would need to be treated for 5.4 years to prevent 1 stroke, this evidence emerged from one mega-trial. Trial sequential analyses showed that additional trials are unlikely to increase the certainty about the findings of this issue regarding homocysteine-lowering interventions versus placebo.

Implications for research

The association between both the lack of clinical effectiveness and harm of homocysteine-lowering interventions might require further investigation into other homocysteine pathways. There is the need for additional trials comparing homocysteine-lowering interventions combined with antihypertensive medication versus antihypertensive medication, and homocysteine-lowering interventions at high doses versus homocysteine-lowering interventions at low doses. Potential trials should be large and co-operative.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

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

B-PROOF 2015

Study characteristics	
Methods	Parallel design (2 arms Multicentre study: yes (3 centres) Country: The Netherlands Follow-up period (years): 2
Participants	**Randomised:** 2919 (Original group of 'B-vitamins for the PRevention Of Osteoporotic Fractures') 1. Intervention: 1458 2. Placebo: 1461 The following data belong to subgroup analysis on cardiovascular events (named as 'Vascular sub-group' by trial authors). **Randomised:** 569 1. Intervention: 274 2. Placebo: 295 **Age (Mean SD):** 1. Intervention: 72.5 (5.8) 2. Placebo: 72.5 (5.3) **Gender (male %):** 1. Intervention: 55.9 2. Placebo: 55.5 **Homocysteine levels at baseline (median interquartile range) (µmol/L):** 1. Intervention: 14.2 (13.0 to 16.3) 2. Placebo: 14.3 (13.0 to 16.6) **Self-reported cardiovascular medical history (intervention versus placebo):** 1. MI: 6.1% vs 10.2% 2. Any type CVD: 21.0% vs 21.9%

B-PROOF 2015 *(Continued)*

	3. Cerebrovascular event: 7.5% vs 8.4% 4. Hypercholesterolaemia: 25.1% vs 28.1% 5. Diabetes: 10.8% vs 12.0% **Inclusion criteria:** 1. Age ≥ 65 years 2. Plasma Hcy level between 12.0 µg/mol/L and 50.0 µg/mol/L **Exclusion criteria:** 1. Serum creatinine level >150 µg mol/L 2. Cancer in the past 5 years (excluding non-melanoma skin cancer), 3. High doses of B-vitamins use (intramuscular injections of vitamin B12 and/or folic acid intake > 300 µg/day) 4. Permanent use of a wheel chair
Interventions	1. Experimental: 400 µg folic acid and 500 µg vitamin B12 2. Control: placebo 3. Co-intervention: 600 IU (15 µg) of vitamin D3 daily Treatment duration: two years
Outcomes	Outcomes related to vascular subgroup analysis: 1. Any type of CVD: MI, angina pectoris, heart failure, cardiac valvular disease, or arrhythmia. 2. Adverse events
Notes	1. Identifier: Netherlands Trial Register NTR 1333 and NCT00696514 2. Conducted between August 2008 and March 2011 3. A priori sample estimation: yes 4. Financial disclosures: Two authors declared to have received an unconditional grant of Merck and Co for vitamin D assessment in Longitudinal Aging Study Amsterdam and one of them received personal fees from Merck and Co and Bristol-Myers Squibb. 5. Other disclosures: none 6. Funding/support: "supported and funded by The Netherlands Organization for Health Research and Development (ZonMw, Grant 6130.0031), the Hague; unrestricted grant from NZO (Dutch Dairy Association), Zoetermeer; Orthica, Almere; NCHA (Netherlands Consortium Healthy Ageing) Leiden/Rotterdam; Ministry of Economic Affairs, Agriculture, and Innovation (Project KB15-004-003), the Hague; Wageningen University, Wageningen; VU University Medical Center, Amsterdam; Erasmus Medical Center, Rotterdam." (Page 408). 7. Rol of sponsor: "The sponsors do not have any role in the design or implementation of the study, data collection, data management, data analysis, data interpretation, or in the preparation, review, or approval of the manuscript" (Page 408).

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote "The random allocation sequence and randomization were generated and performed using SAS 9.2 by an independent research dietician." (Page 402)
Allocation concealment (selection bias)	Low risk	Quote "The random allocation sequence and randomization were generated and performed using SAS 9.2 by an independent research dietician." (Page 402)

B-PROOF 2015 *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote "Intervention and placebo tablets were indistinguishable in taste, smell, and appearance. Both the participants and all researchers and research assistants were blinded to the study treatment" (Page 402)
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Insufficient information on blinding of outcome assessment to judge as 'high' or 'low' risk
Incomplete outcome data (attrition bias) All outcomes	High risk	Follow-up: Experimental group: 94.8% (260/274) Control: 80.3% (237/295) Imbalance between comparison groups: 14.5%
Selective reporting (reporting bias)	Low risk	It is clear that the published reports relevant clinical outcomes
Other bias	Low risk	Other sources of bias not identified

BVAIT 2009

Study characteristics	
Methods	Parallel design Multicentre study: yes Country: USA 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 **Randomised:** 506 (254 vitamins versus 252 placebo) **Age (years):** 1. Overall: 61.4 2. B vitamins group: 61.7 (10.1) 3. Placebo group: 61.1 (9.6) **Gender (men):** 1. Overall: 61% 2. B vitamins group: 61% 3. Placebo group: 61% **Inclusion criteria:** 1. Men and postmenopausal women 40 years old 2. Fasting tHcy 8.5 mol/L 3. No clinical signs/symptoms of CVD **Exclusion criteria:** 1. Fasting triglycerides > 5.64 mmol/L (500 mg/dL) 2. Diabetes mellitus or fasting serum glucose > 6.99 mmol/L (126 mg/dL) 3. Systolic blood pressure ≥ 160 mm Hg and/or diastolic blood pressure ≥ 100 mm Hg

BVAIT 2009 *(Continued)*

4. Untreated thyroid disease
5. Creatinine clearance < 70 mL/min
6. Life-threatening illness with prognosis 5 years
7. Five alcoholic drinks daily

Interventions	1. HLI-intervention: folic acid (5 mg), vitamin B12 (0.4 mg) and vitamin B6 (50 mg, daily supplementation 2. Control: placebo 3. Treatment duration: initial 2.5-year treatment period was extended on average 1 to 2 years
Outcomes	**Primary:** Rate of change in the right distal carotid artery intima media thickness **Secondary:** Changes in calcium in the coronary arteries and abdominal aorta **Safety:** 1. Deaths 2. Cardiovascular events 3. Cerebrovascular events 4. Arterial revascularisation procedures 5. Cancers 6. Occurrence of white blood cell count below the laboratory normal limit (4000 cells/µL)
Notes	1. Identifier: NCT00114400 2. Conducted between 6 November 2000 and 1 June 2006 3. A priori sample estimation: yes 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) 1. Financial disclosures: not reported 2. Other disclosures: none 3. 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 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) 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)	Low risk	Quote: "...imaging specialists, ... were masked to treatment assignment." (page 731). & "Scans were analyzed without knowledge of treatment as-

BVAIT 2009 *(Continued)*

All outcomes		signment using validated calcium scoring software" (for secondary outcome)" (page 731) Comments: the main outcomes were to assess the impact of the HLI on reduction of subclinical atherosclerosis progression
Incomplete outcome data (attrition bias) All outcomes	Low risk	**B vitamins group** 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) 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) **Placebo group** 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) 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) **Evaluable included in analysis:** 1. B vitamins group: 97.6% (248/254) 2. Placebo group: 96% (242/252) 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	Other sources of bias not identified

CHAOS 2002

Study characteristics	
Methods	Parallel design Multicentre study Follow-up period: mean of 1.7 years
Participants	**Randomised:** 1882 participants randomised (folic acid: 942 versus placebo: 940 participants) **Gender:** not reported **Age:** not reported **Homocysteine levels at baseline:** (treatment group) (µmol/L): 11.2 (6.9 µmol/L) **Inclusion criteria:** (1 of the following): 1. Positive coronary angiogram 2. Admission with MI or unstable angina **Exclusion criteria:** not reported

CHAOS 2002 *(Continued)*

Interventions	1. Intervention: folic acid 5 mg per day 2. Control: placebo in addition to usual drugs Treatment duration: 2 years
Outcomes	Composite outcome: MI, revascularisation, death from cardiovascular cause
Notes	1. Sponsors: not available 2. Other: data not yet fully published. Results in the table correspond to conference proceedings 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 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) All outcomes	Unclear risk	Described as double-blinded. However, the information was obtained from the final report (abstract) Insufficient information to permit judgement of 'Low risk' or 'High risk'
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Described as double-blinded. However, the information was obtained from the final report (abstract) Insufficient information to permit judgement of 'Low risk' or 'High risk'
Incomplete outcome data (attrition bias) 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
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

CSPPT 2015

Study characteristics

Methods	Parallel design Multicentre study (32 communities in Jiangsu and Anhui provinces in China) Country: China Run-in treatment: three weeks taken enalapril (10 mg) oral daily. Follow-up period: 5 year

CSPPT 2015 *(Continued)*

Participants	**Randomised:** 20,702 adults with hypertension without history of stroke or MI 1. Enalapril plus folic acid: 10348 2. Enalapril: 10354 **Age, mean (SD) years:** 1. Enalapril plus folic acid: 60.0 (7.5) 2. Enalapril: 60.0 (7.6) **Gender (male):** 1. Enalapril plus folic acid: 41.0 2. Enalapril: 41.1 **Homocysteine, median (IQR), µmol/L:** 1. Enalapril plus folic acid: 12.5 (10.5-15.5) 2. Enalapril: 12.5 (10.5-15.5) **Inclusion criteria:** 1. Men and women aged 45 to 75 years 2. Hypertension (≥140 mm Hg/≥ 90 mm Hg) 3. Taking an antihypertensive medication **Exclusion criteria:** 1. History of physician diagnosed stroke, MI, heart failure, coronary revascularisation, or congenital heart disease
Interventions	1. Experimental: enalapril, 10 mg and folic acid (0.8 mg) single-pill fixed combination 2. Control: enalapril maleate (10 mg) (Lameiya,Yabao Pharmaceutical) Treatment duration (median 4.5 years)
Outcomes	**Primary:** 1. First stroke **Secondary:** 1. First ischaemic stroke 2. First haemorrhagic stroke 3. MI 4. Composite of cardiovascular events: of cardiovascular death, MI, and stroke 5. All-cause death **Other outcome measures (Source:** NCT00794885) 1. Malignant tumours (Neoplasms benign, malignant and unspecified)
Notes	1. Sample size estimation a priori: yes 2. Clinicaltrials.gov Identifier: NCT00794885 3. Trial conduction date: 19 May, 2008, to 24 August, 2013 4. Goverments and academic centres sponsor: yes 5. Drug company sponsor: yes (Shenzhen AUSA Pharmed Co Ltd) 6. Role of sponsor: "The funding organizations/sponsor participated in the study design but had no role in the conduct of the study; collection, management, analysis, and interpretation of the data;

CSPPT 2015 *(Continued)*

preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication." (Page E10).

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote "Randomization was performed centrally by means of 4 randomization tables: 1was a randomization of drug code and treatment allocation, and the other 3 were MTHFR C677T genotype–specific randomized sequences with a fixed block size of 4."
Allocation concealment (selection bias)	Unclear risk	Quote "All study investigators and participants were blinded to the randomization procedure and the treatment assignments." Insufficient information to permit judgement of 'Low risk' or 'High risk' Trial authors did not describe procedure to guarantee an adequate allocation concealment.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote "Both types of tablets were concealed in a single capsule formulation and were identical in appearance, size, color, and taste"
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote "Both types of tablets were concealed in a single capsule formulation and were identical in appearance, size, color, and taste"
Incomplete outcome data (attrition bias) All outcomes	Low risk	**Lost to follow-up:** 1. Overall: 0.32% (67/20702) 2. Experimental: 0.30% (32/10348) 3. Control: 0.33% (35/10354) Trial authors described reasons
Selective reporting (reporting bias)	Low risk	No bias identified
Other bias	High risk	Industry bias

FOLARDA 2004

Study characteristics

Methods	Parallel design Multicentre study Country: The Netherlands Follow-up period: 1 year
Participants	**Randomised:** 283 randomised participants (folic acid: 140 versus standard care: 143) **Gender (% men): folic acid:** 69% versus standard care: 70% **Age (mean):** folic acid: 59 years versus standard care: 59

FOLARDA 2004 *(Continued)*

Homocysteine levels at baseline: not reported

Inclusion criteria (1 of the following):

1. MI
2. Total cholesterol value at admission or within 24 hours after onset of symptoms: > 6.5 µmol/L (251 mg/dL)
3. Elevation of CK-MB at least 2 times upper the limit of normal function
4. Markedly increased chest pain lasting more than 30 minutes or classical ECG changes

Exclusion criteria:

1. Age under 18 years,
2. Use of lipid-lowering agents within the previous 3 months
3. High triglyceride levels > 4.5 µmol/L
4. Known familial dyslipidaemia
5. Low vitamin B12 levels
6. Hyperhomocysteinaemia (total plasma homocysteine > 18 µmol/L) or a known disturbed methionine loading test (total plasma homocysteine > 47 µmol/L)
7. Severe renal failure (serum creatinine > 180 µmol/L)
8. Hepatic disease
9. Severe heart failure (New York Heart Association class IV)
10. Scheduled percutaneous coronary intervention or coronary artery bypass graft operation

Interventions	**Intervention:** Folic acid: 5 mg per day 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. participants 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) **Control:** Standard care: 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) **Treatment duration:** 1 year
Outcomes	1. Cardiovascular death (sudden death, fatal recurrent MI, fatal stroke and other cardiovascular deaths) 2. Non-cardiovascular death 3. Recurrent MI 4. Recurrent ischaemia requiring hospitalisation or revascularisation
Notes	1. Study phase: III 2. 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 participants, assuming a 1-year event rate of 30%. These numbers resulted in an estimation of 120 participants per group. Analyses conducted on ITT basis 3. Sponsors: AstraZeneca, The Netherlands, Working Group on Cardiovascular research, The Netherlands. One author is an Established Investigator of the Netherlands Heart Foundation 4. Other: author did not perform homocysteine-level measures during the study

Risk of bias

Bias	Authors' judgement	Support for judgement

FOLARDA 2004 *(Continued)*

Random sequence generation (selection bias)	Unclear risk	Quote: "patients were randomised..." 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) All outcomes	High risk	Quote: "... treatment with open label folic acid [...] or not"
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "An Independent Data and Safety Monitoring Committee adjudicated all major clinical events."
Incomplete outcome data (attrition bias) All outcomes	High risk	23 participants 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
Other bias	Unclear risk	Insufficient information to permit judgement of 'Low risk' or 'High risk'

GOES 2003

Study characteristics	
Methods	Parallel design Single-centre study Country: The Netherlands Follow-up period: 1 year
Participants	**Rndomised:** 593 randomised participants (folic acid: 300 versus standard care: 293) **Gender (% men):** folic acid: 76% versus standard care: 80% **Age (mean SD):** folic acid: 64.9 (9.9) versus standard care: 65.5 (9.7) **Homocysteine levels at baseline:** not reported **Inclusion criteria:** 1. MI 2. Coronary artery lesions (> 60%) on coronary angiography 3. Percutaneous coronary intervention 4. Coronary artery bypass graft surgery 5. Participants had to be stable, with no invasive vascular procedures scheduled 6. Statin therapy for at least 3 months 7. Taking any form of vitamin B-containing medication, regularly or sporadically **Exclusion criteria:** 1. Age < 18 years

GOES 2003 *(Continued)*

	2. History of low vitamin B12 levels 3. Therapy for hyperhomocysteinaemia 4. Severe renal failure, or any other treatment for renal disease 5. Hepatic disease 6. Severe heart failure (New York Heart Association functional class IV) 7. Serious illness that would exclude follow-up time of at least 3 years
Interventions	1. Intervention: folic acid: 0.5 mg per day 2. Control group: standard care 3. 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 4. Treatment duration: not reported
Outcomes	**Primary (composite):** 1. Composite: vascular death (sudden death, fatal recurrent MI, fatal stroke and other cardiovascular deaths) 2. Non-cardiovascular death 3. Recurrent acute coronary syndromes 4. Invasive coronary procedures 5. Cerebrovascular accident or transient ischaemic attack 6. Any other vascular surgery (carotid endarterectomy, abdominal aneurysmectomy, or peripheral vascular surgery including limb amputation for vascular reasons) **Secondary:** 1. Hospitalisation for unstable angina
Notes	1. Study phase: III 2. 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 3. Sponsors: trial with public funding (Stichting Paracard) 4. 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) All outcomes	High risk	Quote: "... treatment with open label folic acid [...] or standard care."
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "Adjudication of all clinical events was performed by an independent end point monitoring committee unaware of treatment arm."

GOES 2003 *(Continued)*

Incomplete outcome data (attrition bias) 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

Study characteristics	
Methods	Parallel design Multicentre international study (13 countries; 145 centres) Follow-up period: 5 years
Participants	**Randomised:** 5522 patients randomised (vitamin: 2758 versus placebo group: 2764 patients) **Gender (% men):** vitamin: 71.1% versus placebo: 72.4% **Age (mean SD):** vitamin: 68.8 (7.1) versus placebo: 68.9 (6.8) **Homocysteine level at baseline:** 12.2 µmol/L (1.6 mg/L) **Inclusion criteria:** 1. Men and women aged > 55 years 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 USA) and countries without mandatory folate fortification (Brazil, western Europe and Slovakia) **Exclusion criteria:** 1. Patients taking vitamin supplements containing more than 0.2 mg of folic acid per day
Interventions	**Intervention:** 1. Multivitamin therapy with 2.5 mg of folic acid, 50 mg of vitamin B6 and 1 mg of vitamin B12 per day **Control:** 1. Matching placebo daily **Treatment duration:** 5 years
Outcomes	**Primary outcome (composite):** 1. Death from cardiovascular causes, MI, stroke 2. Secondary outcomes: 3. Total ischaemic events (composite of death from cardiovascular causes, MI, stroke, hospitalisation for unstable angina and revascularisation) 4. Death from any cause 5. Hospitalisation for unstable angina or congestive heart failure 6. Revascularisation 7. Incidence and death for cancer

HOPE-2 2006 *(Continued)*

	8. Other outcomes: transient ischaemic attacks, venous thromboembolic events, fractures
Notes	1. Study phase: III, registered (ClinicalTrials.gov number NCT00106886) 2. 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 3. 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"
Allocation concealment (selection bias)	Low risk	Centralised telephone randomisation (accessible 24 hours a day)
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote: "All study investigators, personnel, and participants were unaware of the randomization procedure and the treatment assignments." Vitamins manufactured to be indistinguishable in colour, weight or ability to be dissolved in water
Blinding of outcome assessment (detection bias) All outcomes	Low risk	This trial assessed objective outcomes
Incomplete outcome data (attrition bias) All outcomes	Low risk	21 patients in the treatment group and 16 in the placebo group did not complete the study 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	Other sources of bias not identified

Li 2015a

Study characteristics

Methods	Parallel design Multicentre study: not stated Country: China Follow-up period: 5 years
Participants	1. Elderly females with hypertensive emergencies and homocysteine (Hcy) (>10 mol/L) 2. Randomised: 319 1. High-dose folic acid: 144 2. Low-dose folic acid: 136 **Age:** ≥ 65 year

Li 2015a *(Continued)*

	Gender: only female.
Interventions	1. Intervention: high-dose folic acid: 0.8 mg/day 2. Control: low-dose folic acid: 0.4 mg/day 3. Co-intervention: salt restriction (≤5 g/day) and vitamin B12 500 µg/day.
Outcomes	1. Ischaemic stroke 2. Orthostatic hypotension
Notes	1. Trial conduction date: June 2006 to June 2009 2. Sponsors: not available 3. Other: data not yet fully published. Results in the table correspond to conference proceedings

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Described as randomised Insufficient information about the random sequence generation 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	Described as randomised Insufficient information about the allocation concealment 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) All outcomes	Unclear risk	Insufficient information to permit judgement of 'Low risk' or 'High risk'
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Insufficient information to permit judgement of 'Low risk' or 'High risk'
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Insufficient information to permit judgement of 'Low risk' or 'High risk'
Selective reporting (reporting bias)	High risk	Trial reported no information clinical relevant outcomes such as MI, mortality or harms.
Other bias	Unclear risk	Insufficient information to assess whether an important risk of bias exists

NORVIT 2006

Study characteristics

Methods	Parallel design Multicentre study Country: Norway

NORVIT 2006 *(Continued)*

	Follow-up period: 3.5 years
Participants	**Randomised:** 3749 patients randomised (folic acid, vitamins B6 and B12: 937 versus folic acid, vitamin B12: 935 versus vitamin B6: 934 versus placebo: 943) **Gender (% men):** Folic acid, vitamins B6 and B12: 73% Folic acid, vitamin B12: 74% Vitamin B6: 73% Placebo: 75% **Age (mean SD, years):** Folic acid, vitamins B6 and B12: 63.6 (11.9) Folic acid, vitamin B12: 63.2 (11.6) Vitamin B6: 62.5 (11.7) Placebo: 62.6 (11.4) **Inclusion criteria:** 1. Men and women aged 30 to 85 years, 2. History of acute MI within 7 days before randomisation **Exclusion criteria:** 1. Co-existing disease associated with a life expectancy < 4 years 2. Prescribed treatment with B vitamins or untreated vitamin B deficiency 3. Inability to follow the protocol, as judged by the investigator
Interventions	**Intervention:** 1. Folic acid (group 1): 0.8 mg; vitamin B12: 0.4 mg; vitamin B6: 40 mg per day 2. Folic acid (group 2): 0.8 mg; vitamin B12: 0.4 mg per day 3. Vitamin B6 (group 3): 40 mg per day **Control:** placebo 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 **Treatment duration:** not clearly described
Outcomes	**Primary outcome (composite):** 1. Recurrent MI, stroke and sudden death attributed to CAD **Secondary outcomes:** 1. MI 2. Unstable angina pectoris requiring hospitalisation 3. Coronary revascularisation with percutaneous coronary intervention or coronary artery bypass grafting 4. Stroke 5. Death from any cause Incident cases of cancer
Notes	1. Study phase: III, registered (ClinicalTrials.gov number NCT00266487)

NORVIT 2006 *(Continued)*

2. 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

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

1. 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
Random sequence generation (selection bias)	Unclear risk	No information reported about this domain
Allocation concealment (selection bias)	Low risk	The manufacturer provided central study sites with blocks of medication assigned in numerical order
Blinding of participants and personnel (performance bias) All outcomes	Low risk	All study personnel and participants were unaware of the treatment assignments Vitamins were manufactured to be indistinguishable in colour, weight or ability to be dissolved in water
Blinding of outcome assessment (detection bias) 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) All outcomes	Low risk	11% of patients stopped the medication 94% attended the final visit, but data on mortality were available for the entire sample. Incomplete outcome data for 20 patients 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	Other sources of bias not identified

SEARCH 2010

Study characteristics

Methods	Parallel design Multicentre study (88 sites) Country: UK

SEARCH 2010 *(Continued)*

	Follow-up period: 6.7 ± 1.5 person-years
Participants	Clinical condition: survivors of MI in secondary care hospitals 1. Potential participants invited by mail: 83,237 2. Attended screening visit: 34,780 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)" (page 2487) 4. Randomised: 12,064 (folic acid and B12: 6033 versus placebo: 6031) **Gender (% men):** Men: 10,012 Women: 2052 1. Folic acid and B12: 83% 2. Placebo: 83% **Age (at randomisation):** Mean (SD) age of 64.2 (8.9) years Folic acid and vitamin B12: 1. < 60 years: 31% 2. ≥ 60 years to < 70 years: 40% 3. ≥ 70 years: 29% **Placebo:** 1. < 60 years: 31% 2. ≥ 60 years to < 70 years: 40% 3. ≥ 70 years: 29% **Inclusion criteria:** 1. Men and women 2. Aged 18 to 80 years 3. History of MI 4. Had no clear indication for folic acid 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) **Exclusion criteria:** 1. Chronic liver, renal or muscle disease 2. History of any cancer (except non-melanoma skin cancer) 3. Use of potentially interacting medications
Interventions	1. Intervention: 1 tablet daily containing 2 mg folic acid plus 1 mg vitamin B12 2. Control: placebo 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):** 1. Incidence of first major vascular event, defined as non-fatal MI or death from CHD, fatal or non-fatal stroke, or any arterial revascularisation

SEARCH 2010 *(Continued)*

Secondary outcomes:

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
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)
3. Major vascular events in the presence of one or other of the allocated study simvastatin regimens
4. Major coronary events, defined as non-fatal MI, death from coronary disease, or coronary revascularisation
5. Any type of stroke (excluding transient ischaemic attacks)

Tertiary outcomes:

1. Total and cause-specific mortality (considering vascular and non-vascular causes separately)
2. Vascular mortality excluding the first year after randomisation
3. Coronary and non-coronary revascularisation separately
4. Confirmed haemorrhagic and other strokes separately
5. Pulmonary embolus
6. Total and site-specific cancers
7. Hospitalisations for various other causes
8. Adverse effects of treatment

Notes

Identifier: ISRCTN 74348595

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)

Conducted between September 1998 and June 2008

A priori sample estimation: yes

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)
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)
3. Comment: assumptions for sample size estimation were based on Boushey 1995; Bowman 2007; HSC 2002 and SSSS 1994

1. Financial disclosures: reported
2. 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)
3. 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)
4. 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)

SEARCH 2010 *(Continued)*

Allocation concealment (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)
Blinding of participants and personnel (performance bias) 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) 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) All outcomes	Low risk	Vitamin group: 98.9% (5970/6033) completed follow-up 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	Other sources of bias not identified

SU.FOL.OM3 2010

Study characteristics	
Methods	Parallel design Multicentre study (257 sites) Country: France 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 1. Patients assessed for eligibility: 3374 2. Randomised: 2501 (B vitamins plus omega 3 fatty acids: 620, omega 3 fatty acids: 633, B vitamins: 622, and placebo: 626) 3. Complete follow-up: 2222 (89%) **Gender (% men):** Men: 1987 Women: 514 1. B vitamins plus omega 3 fatty acids: 79.5% 2. Omega 3 fatty acids: 79.2% 3. B vitamins: 79.9% 4. Placebo: 79.2% **Age:** Mean (SD) age of 60.9 (8.8) years. 1. B vitamins plus omega 3 fatty acids: 60.5 (53.9 to 68.9) 2. Omega 3 fatty acids: 60.41 (5.7 to 68.7)

SU.FOL.OM3 2010 *(Continued)*

3. B vitamins: 60.7 (54.7 to 68.3)
4. Placebo: 60.9 (54.5 to 68.1)

Inclusion criteria:

1. Men and women
2. Aged 45 to 80 years
3. History of acute coronary or cerebral ischaemic event within the 12 months before randomisation

Exclusion criteria:

1. Age (< 45 years or > 80 years)
2. Ill-defined diagnosis of CVD
3. Inability or unwillingness to comply with study treatment
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)
5. Individuals with transient ischaemic attacks

Interventions	1. Intervention: 1 tablet daily containing 5-methyltetrahydrofolate (560 µg), vitamin B6 (3 mg) and B12 (20 µg) 2. Control: placebo 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):** 1. First major cardiovascular event: non-fatal MI, ischaemic stroke or death from CVD (including fatal MI, 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) **Secondary outcomes:** 1. Acute coronary syndrome without MI 2. Resuscitation from sudden death 3. Coronary artery bypass surgery 4. Coronary angioplasty 5. Cardiac failure 6. Ventricular arrhythmia 7. Supraventricular arrhythmia 8. Cardiac surgery of any kind, transient ischaemic attack 9. Deep vein thrombosis 10. Pulmonary embolism 11. Carotid surgery or carotid artery angioplasty 12. Peripheral arterial surgery or angioplasty 13. Any vascular procedure 14. Death from all causes
Notes	Identifier: ISRCTN 41926726 Conducted between 1 February 2003 and 1 June 2007 **A priori sample estimation: yes** 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

SU.FOL.OM3 2010 *(Continued)*

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 α value of 0.05." (page 3)

Comment: assumptions were based on Galan et al (HSC 2002; SU.FOL.OM3 2010; Yusuf 2000)

1. Competing interest: reported
2. 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)
3. 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) All outcomes	Low risk	Quote: "Patients, clinicians, trial coordinators, and outcome investigators were blinded to treatment allocation." (page 2) Quote: "treatment capsules for one year (and repeated yearly) in an appropriately labelled package." (page 2)
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "... and outcome investigators were blinded to treatment allocation." (page 2) 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) All outcomes	Low risk	1. B vitamins plus omega 3 fatty acids: 11.8% (547/620) 2. Omega 3 fatty acids: 9.6% (572/633) 3. B vitamins: 12.6% (542/622) 4. Placebo: 10.4% (561/626) 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	Other sources of bias not identified

VISP 2004

Study characteristics	
Methods	Parallel design Country: USA, Canada and Scotland Multicentre international study Follow-up period: 2 years
Participants	3680 randomised (high-dose: 1827 versus low-dose: 1853) **Age (mean SD):** high-dose: 66.4 (10.8) versus low-dose: 66.2 (10.8) **Gender (% men):** high-dose: 62.3% versus low-dose: 62.8% **Inclusion criteria:** 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 2. Total homocysteine level 25th percentile for North American stroke population 3. Age: ≥ 35 years 4. Accessibility for follow-up 5. Agreement to take study medication and not take other multivitamins or pills containing folic acid or vitamin B6 6. Written informed consent **Exclusion criteria:** 1. Potential sources of emboli (atrial fibrillation within 30 days of stroke, prosthetic cardiac valve, intracardiac thrombus or neoplasm, or valvular vegetation) 2. Other major neurological illness that would obscure evaluation of recurrent stroke 3. Life expectancy 2 years 4. Renal failure requiring dialysis 5. Untreated anaemia or untreated vitamin B12 deficiency 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 7. Refractory depression, severe cognitive impairment, or alcoholism or other substance abuse 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 9. Childbearing potential 10. Participation in another trial with active intervention 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** 2.5 mg folic acid; 0.4 mg vitamin B12; 25 mg vitamin B6 per day **Low-dose multivitamin therapy** 20 micrograms folic acid; 6 micrograms vitamin B12; 200 micrograms vitamin B6 per day **Co-interventions:** 1. Risk factor control education 2. Aspirin (325 mg/day) **Duration of treatment:** not described

VISP 2004 *(Continued)*

Outcomes	**Primary outcome:** 1. Recurrent cerebral infarction **Secondary outcomes:** 1. CHD, including: MI requiring hospitalisation; coronary revascularisation; and fatal CHD 2. Death
Notes	1. Study phase: III 2. 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 3. 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)	Low 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 After verification of eligibility participants were assigned in 1 of 20 medication codes
Blinding of participants and personnel (performance bias) 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) All outcomes	Low risk	The primary endpoint was reviewed by a local neurologist and 2 external independent review neurologists
Incomplete outcome data (attrition bias) 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 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	Other sources of bias not identified

VITATOPS 2010

Study characteristics	
Methods	Parallel design Multicentre study: 123 medical centres (20 countries) from 4 continents Follow-up period (median and interquartile range, years): 3.4 (2.0 to 5.5)
Participants	8164 randomised 4089 received folic acid and vitamins B (B6 and B12) 4075 received placebo **Age (mean SD years):** 1. Overall: 62.6 (12.5) 2. Vitamin: 62.5 (12.6) 3. Placebo: 62.6 (12.4) **Gender (men):** 1. Overall: 64% 2. Vitamin: 64% (2614/4089) 3. Placebo: 64% (2604/4075) **Inclusion criteria:** 1. Stroke (ischaemic or haemorrhagic) or transient ischaemic attack (eye or brain), as defined by standard criteria, within the past 7 months 2. Patients with haemorrhagic stroke **Exclusion criteria:** 1. Taking folic acid, vitamin B6, vitamin B12 or a folate antagonist (e.g. methotrexate) 2. Pregnant or women of childbearing potential 3. Patients with limited life expectancy (e.g. because of ill health)
Interventions	**Intervention:** 1. Folic acid: 2 mg/day 2. Vitamin B_6: 25 mg/day 3. Vitamin B_{12}: 0.5 mg/day **Control:** placebo **Co-interventions:** not reported
Outcomes	**Primary outcome (composite):** whichever occurred first 1. Non-fatal stroke 2. Non-fatal MI 3. Death from any vascular causes **Secondary outcomes:** 1. Stroke (non-fatal or fatal) 2. MI (non-fatal or fatal) 3. Death from any vascular cause 4. Death from any cause 5. Revascularisation procedures 6. The composite of non-fatal stroke, non-fatal MI and death from any vascular cause

VITATOPS 2010 *(Continued)*

	7. Revascularisation procedures of the coronary, cerebral or peripheral circulation
Notes	Identifier numbers: NCT00097669 and ISRCTN74743444 Date of study: 19 November 1998 to 31 December 2008 1. 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 2. 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 3. RoI 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) 4. Conflicts of interest: reported 5. Vitamin tablets and matching placebo tablets were supplied by Blackmores, Australia (page 864) 6. 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) All outcomes	Low risk	Quote: "Patients, clinicians, trial coordinators, and outcome investigators were masked to treatment allocation" (page 856) Quote: "had the same colour and coating" (page 856)
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "...and outcome investigators were masked to treatment allocation" (page 856)
Incomplete outcome data (attrition bias) All outcomes	Low risk	Loss to final follow-up: Global: 8.6% (702/8164) B vitamins group: 8.5% (348/4089) Placebo group: 8.7% (354/4075) 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)

VITATOPS 2010 *(Continued)*

Other bias	Low risk	Other sources of bias not identified

WAFACS 2008

Study characteristics	
Methods	Parallel design Multicentre study Country: USA Follow-up period: 7.3 years
Participants	N: 5442 randomised patients (vitamin group: 2721 patients; placebo group: 2721 patients) **Gender:** women health professionals **Age (mean (SD)) years:** Active group: 62.8 (8.8) Control group: 62.8 (8.8) **Inclusion criteria** 1. Women 2. Age: 40 years or older 3. Postmenopausal or had no intention of becoming pregnant 4. History of CVD or had at least 3 cardiac risk factors **Exclusion criteria:** 1. Cancer (excluding non-melanoma skin cancer) within the past 10 years 2. Serious non-CVD 3. Warfarin or other anticoagulants use
Interventions	**Intervention:** Folic acid: 2.5 mg; vitamin B12: 1 mg; vitamin B6: 50 mg per day **Control:** Matching placebo per day **Co-interventions:** vitamin C, vitamin E, ß-carotene **Treatment duration:** not clearly reported
Outcomes	**Primary (composite):** 1. Incident MI, stroke, coronary revascularisation procedures (coronary artery bypass grafting or percutaneous coronary intervention) and cardiovascular mortality **Secondary:** 1. MI rate 2. Stroke rate 3. Total CHD events (MI, coronary revascularisation and death from CHD)
Notes	1. Study phase: III, registered (ClinicalTrials.gov number NCT00000541)

WAFACS 2008 *(Continued)*

2. 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)

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

1. 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
2. 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)
3. 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
4. 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) All outcomes	Low risk	All study investigators, personnel and participants were unaware of the participants' treatment assignments Patients were sent packs containing medication or matching placebos identical in appearance An independent committee monitored the "safety and overall quality and scientific integrity" of the trial, which was blinded to treatment assignment All the information was supplied by Nancy Cook (WACS statistician, 23 June 2008)
Blinding of outcome assessment (detection bias) 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 All the information was supplied by Nancy Cook (WACS statistician, 23 June 2008) Comments: this trial had objective outcomes
Incomplete outcome data (attrition bias) 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

WAFACS 2008 *(Continued)*

Other bias	Low risk	Other sources of bias not identified

WENBIT 2008

Study characteristics	
Methods	Parallel design Multicentre study Country: Norway 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):** 1. Folic acid, vitamins B6 and B12: 81.2% 2. Folic acid, vitamin B12: 80.4% 3. Vitamin B6: 80.2% 4. Placebo: 76.5% **Age (mean SD, years):** 1. Folic acid, vitamins B6 and B12: 61.7 (10.3) 2. Folic acid, vitamin B12: 61.3 (10.0) 3. Vitamin B6: 61.4 (9.7) 4. Placebo: 62.0 (9.9) **Inclusion criteria:** 1. Age: 18 years or older 2. Undergoing coronary angiography for suspected CAD and/or aortic valve stenosis at the 2 university hospitals in western Norway **Exclusion criteria:** 1. Unavailability for follow-up 2. Participation in other trials 3. History of alcohol abuse, serious mental illness or cancer
Interventions	**Intervention:** 1. Folic acid (group 1): 0.8 mg; vitamin B12: 0.4 mg; vitamin B6: 40 mg per day 2. Folic acid (group 2): 0.8 mg; vitamin B12: 0.4 mg per day 3. Vitamin B6 (group 3): 40 mg per day **Control: placebo** **Co-interventions:** statins, insulin, aspirin, clopidogrel, beta-blockers, ACE inhibitors/ARBs, calcium channel blockers, loop diuretics, oral antidiabetics, medication for chronic obstructive pulmonary disease **Duration of treatment:** not described
Outcomes	**Primary outcome (composite):**

WENBIT 2008 *(Continued)*

1. All-cause death, non-fatal acute MI, acute hospitalisation for unstable angina pectoris and non-fatal thromboembolic stroke

Secondary outcomes:

1. Acute MI
2. Acute hospitalisation for angina pectoris
3. Stable angina pectoris with angiographically verified progression
4. Myocardial revascularisation procedures
5. Stroke
6. Incident cases of cancer

Notes

1. Study phase: III, registered (ClinicalTrials.gov number NCT00354081)
2. 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
3. Sponsors: the Advanced Research Program and Research Council of Norway, the Norwegian Foundation for Health and Rehabilitation, the Norwegian Heart and Lung Patient Organisation, the Norwegian Ministry of Health and Care Services, the Western Norway Regional Health Authority, the Department of Heart Disease at Haukeland University Hospital, Locus for Homocysteine and Related Vitamins at the University of Bergen, Locus for Cardiac Research at the University of Bergen, the Foundation to Promote Research Into Functional Vitamin B12 Deficiency, Bergen, Norway, and Alpharma Inc, Copenhagen, Denmark
4. 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
5. 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
6. 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) 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) 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) All outcomes	Low risk	Quote: "end-points committees were unaware of the treatment allocation"
Incomplete outcome data (attrition bias) 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

WENBIT 2008 *(Continued)*

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	Other sources of bias not identified

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
IQR: interquartile range
ITT: intention-to-treat
IU: international units
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
Bahmani 2014	Randomised clinical trial that did not assess patient-oriented outcomes and excluded the pre-defined outcomes for this Cochrane review
Bailey 2015	Observational study
Baszczuk 2014	Narrative review
Baszczuk 2015	Non randomised clinical trial
Bazzano 2006	Systematic review
Bobak 2014	Observational study
Clarke 2010	Systematic review
Cui 2010	Observational study
Debreceni 2014	Narrative review
Dell'edera 2013	Non randomised clinical trial
Deshmukh 2010	Randomised clinical trial that did not assess patient-oriented outcomes and excluded the pre-defined outcomes for this Cochrane review
Dong 2015	Network meta-analysis
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

Study	Reason for exclusion
Ebbing 2009	Combined analyses of NORVIT 2006 and WENBIT 2008
Ebbing 2009a	Combined analyses of NORVIT 2006and WENBIT 2008
FINEST 2006	Randomised clinical trial with follow-up of less than 1 year
Goel 2015	Comment on CSPPT 2015
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
Huang 2012	Systematic review
Huang 2015	Randomised clinical trial that did not assess patient-oriented outcomes and excluded the pre-defined outcomes for this Cochrane 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
Li 2014	Systematic review
Li 2015	Systematic review
Liu 2014	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
Méndez-González 2010	Narrative review
Miller 2010	Systematic review
Mishchenko 2015	Pharmacoeconomic study
Moghaddasi 2010	Case-control study
Ntaios 2009	Narrative review

Study	Reason for exclusion
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
Park 2016	Systematic review
Qin 2014	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
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
Wang 2015a	Randomised clinical trial that did not assess patient-oriented outcomes and excluded the pre-defined outcomes for this Cochrane review
Wang 2015b	Systematic review
Wierzbicki 2007	Narrative review
Wise 2015	Comment on CSPPT 2015
Yang 2012	Systematic review
Yi 2014	Systematic review
Zappacosta 2013	Randomised clinical trial that did not assess patient-oriented outcomes and excluded the pre-defined outcomes for this Cochrane review
Zeng 2015	Systematic review
Zhang 2009	Systematic review
Zhang 2013	Systematic review

Study	Reason for exclusion
Zhang 2014	Systematic review
Zhou 2011	Systematic review

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

NCT01956786

Study name	Efficacy of amlodipine-folic acid tablets on reduction of blood pressure and plasma homocysteine
Methods	1. Allocation: randomised. 2. Endpoint classification: safety/efficacy study. 3. Intervention model: parallel assignment. 4. Masking: double-blind (participant, caregiver, investigator, outcomes assessor). 5. Primary purpose: treatment. 6. Study phase: a. Phase 2 b. Phase 3
Participants	Age: 18 years to 75 years. Gender: both. Inclusion criteria: 1. Aged 18-75 years. 2. Sedentary systolic blood pressure between 140 mmHg and 180 mmHg, and/or sedentary diastolic blood pressure between 90 mmHg and 110 mmHg. 3. Plasma homocysteine ≥ 10 μmol/L. 4. Angiotension-converting enzyme inhibitor Intolerance. 5. Signed the written informed consent. Exclusion criteria: 1. Pregnant women or women within lactation period. 2. Hypersensitive to calcium channel blocker or folic acid. 3. Easily hypersensitiveness; 4. Diagnosed secondary hypertension or sceptical secondary hypertension. 5. Severe hypertension (sedentary systolic blood pressure greater than or equal to 180 mmHg and/or sedentary diastolic blood pressure greater than or equal to 110 mmHg). 6. Severe diseases: cardiovascular system, alimentary system, urinary system, endocrine system, respiratory system, neuropsychiatric system, and others such as malignant tumour, malnutrition, hematogenesis dysfunction. 7. Obviously abnormal laboratory examination or signs. 8. Taking other antihypertensive drugs and unwilling to stop. 9. Taking folic acid or other Vitamin B groups and unwilling to stop. 10. Participation in any drug trial not yet approved within 4 weeks before the first visit.
Interventions	1. Experimental (low-dose group): amlodipine-folic acid tablet: 5 mg amlodipine combined with 0.4 mg folic acid, once daily. 2. Experimental (high-dose group): amlodipine-folic acid tablet: 5 mg amlodipine combined with 0.8 mg folic acid,once daily. 3. Control: amlodipine: 5 mg, once daily.

NCT01956786 *(Continued)*

Outcomes	1. Primary: combined effective rate of blood pressure and plasma homocysteine reduction. 2. Secondary: a. Blood pressure reduction b. Plasma homocysteine reduction
Starting date	September 2013.
Contact information	Wang Jiguang, MD. Ruijin Hospital, Shanghai Jiao Tong University.
Notes	1. Official title: efficacy of amlodipine-folic acid tablets on reduction of blood pressure and plasma homocysteine in patients with mild to moderate hypertension, hyperhomocysteinaemia and angiotension-converting enzyme inhibitor Intolerance. 2. Sponsor: Shenzhen Ausa Pharmed Co.,Ltd. 3. Collaborators: a. Ruijin Hospital b. Second Affiliated Hospital of Nanchang University c. Xuzhou Medical College 1. Information provided by (Responsible party): a. Shenzhen Ausa Pharmed Co.,Ltd 1. Estimated enrolled: 540. 2. Listed location countries: China.

DATA AND ANALYSES

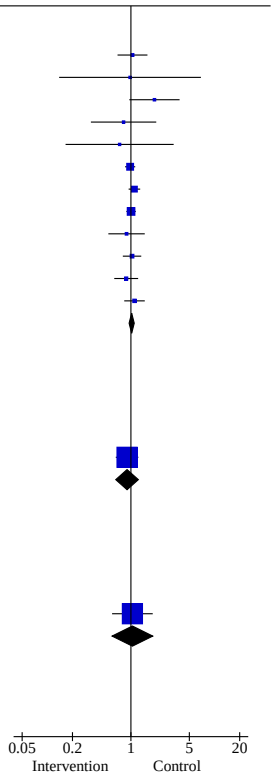
Comparison 1. Homocysteine-lowering treatment versus other (any comparisons)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1 Myocardial infarction	14		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
1.1.1 Homocysteine-lowering versus placebo	12	46699	Risk Ratio (M-H, Random, 95% CI)	1.02 [0.95, 1.10]
1.1.2 Homocysteine-lowering treatment at high dose versus low dose	1	3649	Risk Ratio (M-H, Random, 95% CI)	0.90 [0.66, 1.23]
1.1.3 Homocysteine-lowering treatment (folic acid) plus antihypertensive therapy (enalapril) versus antihypertensive therapy (enalapril)	1	20702	Risk Ratio (M-H, Random, 95% CI)	1.04 [0.60, 1.82]
1.2 Stroke	13		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
1.2.1 Homocysteine-lowering treatment versus placebo	10	44224	Risk Ratio (M-H, Random, 95% CI)	0.90 [0.82, 0.99]
1.2.2 Homocysteine-lowering treatment at high dose versus low dose	2	3929	Risk Ratio (M-H, Random, 95% CI)	0.90 [0.66, 1.22]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.2.3 Homocysteine-lowering treatment (folic acid) plus antihypertensive therapy (enalapril) versus antihypertensive therapy (enalapril)	1	20702	Risk Ratio (M-H, Random, 95% CI)	0.79 [0.68, 0.93]
1.3 First unstable angina pectoris episode requiring hospitalisation	4	12644	Risk Ratio (M-H, Random, 95% CI)	0.98 [0.80, 1.21]
1.4 Death from any cause	13		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
1.4.1 Homocysteine-lowering treatment versus placebo	11	44817	Risk Ratio (M-H, Random, 95% CI)	1.01 [0.96, 1.06]
1.4.2 Homocysteine-lowering treatments at high dose versus low dose	1	3649	Risk Ratio (M-H, Random, 95% CI)	0.86 [0.66, 1.11]
1.4.3 Homocysteine-lowering treatment (folic acid) plus antihypertensive therapy (enalapril) versus antihypertensive therapy (enalapril)	1	20702	Risk Ratio (M-H, Random, 95% CI)	0.94 [0.81, 1.10]
1.5 Serious adverse events (cancer)	9		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
1.5.1 Homocysteine-lowering versus placebo	8	35788	Risk Ratio (M-H, Random, 95% CI)	1.07 [1.00, 1.14]
1.5.2 Homocysteine-lowering treatment (folic acid) plus antihypertensive therapy (enalapril) versus antihypertensive therapy (enalapril)	1	20243	Risk Ratio (M-H, Random, 95% CI)	0.96 [0.71, 1.31]
1.6 Adverse events (serious and non-serious) excluding cancer	3	13802	Risk Ratio (M-H, Random, 95% CI)	1.02 [0.88, 1.19]
1.6.1 Homocysteine-lowering versus placebo	3	13802	Risk Ratio (M-H, Random, 95% CI)	1.02 [0.88, 1.19]

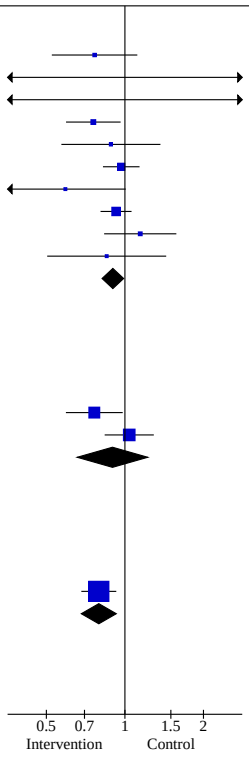
Analysis 1.1. Comparison 1: Homocysteine-lowering treatment versus other (any comparisons), Outcome 1: Myocardial infarction

Study or Subgroup	Intervention Events	Intervention Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
1.1.1 Homocysteine-lowering versus placebo						
B-PROOF 2015	45	1461	43	1458	2.9%	1.04 [0.69 , 1.58]
BVAIT 2009	2	248	2	242	0.1%	0.98 [0.14 , 6.87]
CHAOS 2002	23	942	12	940	1.0%	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	25.4%	0.98 [0.85 , 1.13]
NORVIT 2006	534	2806	163	943	19.5%	1.10 [0.94 , 1.29]
SEARCH 2010	431	6033	429	6031	29.8%	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	7.7%	1.03 [0.80 , 1.33]
WAFACS 2008	65	2721	74	2721	4.6%	0.88 [0.63 , 1.22]
WENBIT 2008	190	2311	58	779	6.2%	1.10 [0.83 , 1.46]
Subtotal (95% CI)		**25051**		**21648**	**100.0%**	**1.02 [0.95 , 1.10]**
Total events:	1788		1290			
Heterogeneity: Tau² = 0.00; Chi² = 6.28, df = 11 (P = 0.85); I² = 0%						
Test for overall effect: Z = 0.58 (P = 0.56)						
1.1.2 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]**
Total events:	72		81			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.67 (P = 0.50)						
1.1.3 Homocysteine-lowering treatment (folic acid) plus antihypertensive therapy (enalapril) versus antihypertensive therapy (enalapril)						
CSPPT 2015	25	10348	24	10354	100.0%	1.04 [0.60 , 1.82]
Subtotal (95% CI)		**10348**		**10354**	**100.0%**	**1.04 [0.60 , 1.82]**
Total events:	25		24			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.15 (P = 0.88)						



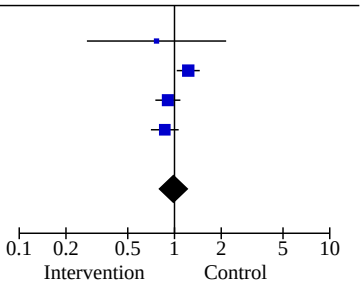
Analysis 1.2. Comparison 1: Homocysteine-lowering treatment versus other (any comparisons), Outcome 2: Stroke

Study or Subgroup	Intervention Events	Intervention Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
1.2.1 Homocysteine-lowering treatment versus placebo						
B-PROOF 2015	46	1461	60	1458	6.0%	0.77 [0.52 , 1.12]
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	13.8%	0.76 [0.59 , 0.96]
NORVIT 2006	71	2806	27	943	4.5%	0.88 [0.57 , 1.37]
SEARCH 2010	277	6033	286	6031	26.7%	0.97 [0.82 , 1.14]
SU.FOL.OM3 2010	21	1242	36	1259	3.1%	0.59 [0.35 , 1.01]
VITATOPS 2010	360	4089	388	4075	34.2%	0.92 [0.81 , 1.06]
WAFACS 2008	79	2721	69	2721	8.3%	1.14 [0.83 , 1.57]
WENBIT 2008	48	2311	19	779	3.2%	0.85 [0.50 , 1.44]
Subtotal (95% CI)		**23809**		**20415**	**100.0%**	**0.90 [0.82 , 0.99]**
Total events:	1014		1034			
Heterogeneity: Tau² = 0.00; Chi² = 9.81, df = 9 (P = 0.37); I² = 8%						
Test for overall effect: Z = 2.19 (P = 0.03)						
1.2.2 Homocysteine-lowering treatment at high dose versus low dose						
Li 2015a	59	144	73	136	48.0%	0.76 [0.59 , 0.98]
VISP 2004	152	1814	148	1835	52.0%	1.04 [0.84 , 1.29]
Subtotal (95% CI)		**1958**		**1971**	**100.0%**	**0.90 [0.66 , 1.22]**
Total events:	211		221			
Heterogeneity: Tau² = 0.04; Chi² = 3.53, df = 1 (P = 0.06); I² = 72%						
Test for overall effect: Z = 0.69 (P = 0.49)						
1.2.3 Homocysteine-lowering treatment (folic acid) plus antihypertensive therapy (enalapril) versus antihypertensive therapy (enalapril)						
CSPPT 2015	281	10348	354	10354	100.0%	0.79 [0.68 , 0.93]
Subtotal (95% CI)		**10348**		**10354**	**100.0%**	**0.79 [0.68 , 0.93]**
Total events:	281		354			
Heterogeneity: Not applicable						
Test for overall effect: Z = 2.93 (P = 0.003)						



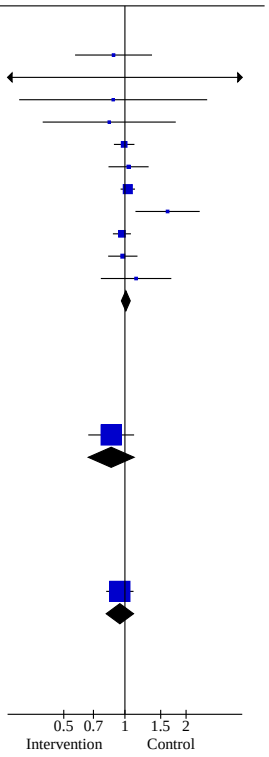
Analysis 1.3. Comparison 1: Homocysteine-lowering treatment versus other (any comparisons), Outcome 3: First unstable angina pectoris episode requiring hospitalisation

Study or Subgroup	Intervention Events	Intervention Total	Control Events	Control Total	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		468			
Heterogeneity: Tau² = 0.03; Chi² = 8.72, df = 3 (P = 0.03); I² = 66%						
Test for overall effect: Z = 0.16 (P = 0.87)						
Test for subgroup differences: Not applicable						



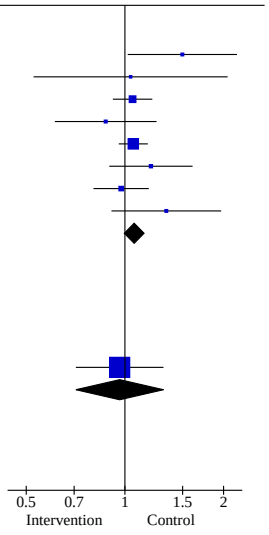
Analysis 1.4. Comparison 1: Homocysteine-lowering treatment versus other (any comparisons), Outcome 4: Death from any cause

Study or Subgroup	Intervention Events	Intervention Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
1.4.1 Homocysteine-lowering treatment versus placebo						
B-PROOF 2015	37	1461	42	1458	1.3%	0.88 [0.57 , 1.36]
BVAIT 2009	0	248	2	242	0.0%	0.20 [0.01 , 4.04]
FOLARDA 2004	6	140	7	143	0.2%	0.88 [0.30 , 2.54]
GOES 2003	12	300	14	293	0.4%	0.84 [0.39 , 1.78]
HOPE-2 2006	470	2758	475	2764	18.7%	0.99 [0.88 , 1.11]
NORVIT 2006	276	2806	89	943	4.9%	1.04 [0.83 , 1.31]
SEARCH 2010	983	6033	951	6031	37.7%	1.03 [0.95 , 1.12]
SU.FOL.OM3 2010	72	1242	45	1259	1.9%	1.62 [1.13 , 2.33]
VITATOPS 2010	614	4089	633	4075	24.1%	0.97 [0.87 , 1.07]
WAFACS 2008	250	2721	256	2721	9.1%	0.98 [0.83 , 1.15]
WENBIT 2008	101	2311	30	779	1.6%	1.13 [0.76 , 1.69]
Subtotal (95% CI)		**24109**		**20708**	**100.0%**	**1.01 [0.96 , 1.06]**
Total events:	2821		2544			
Heterogeneity: Tau² = 0.00; Chi² = 9.99, df = 10 (P = 0.44); I² = 0%						
Test for overall effect: Z = 0.41 (P = 0.68)						
1.4.2 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		117			
Heterogeneity: Not applicable						
Test for overall effect: Z = 1.17 (P = 0.24)						
1.4.3 Homocysteine-lowering treatment (folic acid) plus antihypertensive therapy (enalapril) versus antihypertensive therapy (enalapril)						
CSPPT 2015	302	10348	320	10354	100.0%	0.94 [0.81 , 1.10]
Subtotal (95% CI)		**10348**		**10354**	**100.0%**	**0.94 [0.81 , 1.10]**
Total events:	302		320			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.73 (P = 0.47)						



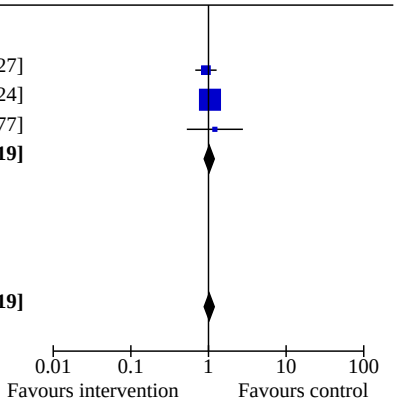
Analysis 1.5. Comparison 1: Homocysteine-lowering treatment versus other (any comparisons), Outcome 5: Serious adverse events (cancer)

Study or Subgroup	Intervention Events	Intervention Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
1.5.1 Homocysteine-lowering versus placebo						
B-PROOF 2015	63	1461	42	1458	3.3%	1.50 [1.02 , 2.20]
BVAIT 2009	16	248	15	242	1.0%	1.04 [0.53 , 2.06]
HOPE-2 2006	358	2758	340	2764	24.8%	1.06 [0.92 , 1.21]
NORVIT 2006	104	2806	40	943	3.8%	0.87 [0.61 , 1.25]
SEARCH 2010	678	6033	639	6031	45.5%	1.06 [0.96 , 1.17]
SU.FOL.OM3 2010	92	1253	77	1259	5.6%	1.20 [0.90 , 1.61]
WAFACS 2008	187	2721	192	2721	12.7%	0.97 [0.80 , 1.18]
WENBIT 2008	123	2311	31	779	3.2%	1.34 [0.91 , 1.97]
Subtotal (95% CI)		**19591**		**16197**	**100.0%**	**1.07 [1.00 , 1.14]**
Total events:	1621		1376			
Heterogeneity: Tau² = 0.00; Chi² = 7.03, df = 7 (P = 0.43); I² = 0%						
Test for overall effect: Z = 1.83 (P = 0.07)						
1.5.2 Homocysteine-lowering treatment (folic acid) plus antihypertensive therapy (enalapril) versus antihypertensive therapy (enalapril)						
CSPPT 2015	79	10119	82	10124	100.0%	0.96 [0.71 , 1.31]
Subtotal (95% CI)		**10119**		**10124**	**100.0%**	**0.96 [0.71 , 1.31]**
Total events:	79		82			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.23 (P = 0.81)						



Analysis 1.6. Comparison 1: Homocysteine-lowering treatment versus other (any comparisons), Outcome 6: Adverse events (serious and non-serious) excluding cancer

Study or Subgroup	Intervention Events	Intervention Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
1.6.1 Homocysteine-lowering versus placebo						
BVAIT 2009	57	248	60	242	22.2%	0.93 [0.68 , 1.27]
SEARCH 2010	253	6033	242	6031	74.6%	1.05 [0.88 , 1.24]
SU.FOL.OM3 2010	12	622	10	626	3.2%	1.21 [0.53 , 2.77]
Subtotal (95% CI)		**6903**		**6899**	**100.0%**	**1.02 [0.88 , 1.19]**
Total events:	322		312			
Heterogeneity: Tau² = 0.00; Chi² = 0.59, df = 2 (P = 0.75); I² = 0%						
Test for overall effect: Z = 0.29 (P = 0.77)						
Total (95% CI)		**6903**		**6899**	**100.0%**	**1.02 [0.88 , 1.19]**
Total events:	322		312			
Heterogeneity: Tau² = 0.00; Chi² = 0.59, df = 2 (P = 0.75); I² = 0%						
Test for overall effect: Z = 0.29 (P = 0.77)						
Test for subgroup differences: Not applicable						

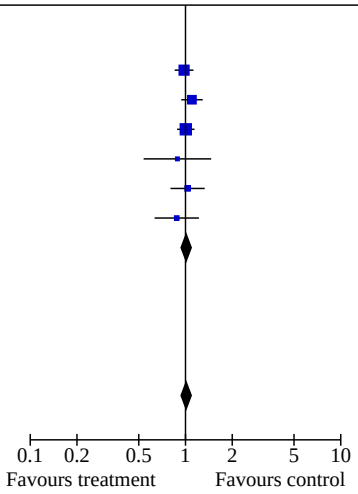


Comparison 2. Homocysteine-lowering treatment versus placebo or standard care (Sensitivity analysis)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.1 Myocardial infarction	6	37442	Risk Ratio (M-H, Random, 95% CI)	1.01 [0.94, 1.09]
2.1.1 Trials with low risk of bias	6	37442	Risk Ratio (M-H, Random, 95% CI)	1.01 [0.94, 1.09]
2.2 Stroke	6	37442	Risk Ratio (M-H, Random, 95% CI)	0.90 [0.80, 1.02]
2.2.1 Trials with low risk of bias	6	37442	Risk Ratio (M-H, Random, 95% CI)	0.90 [0.80, 1.02]
2.3 Death from any cause	7	37932	Risk Ratio (M-H, Random, 95% CI)	1.03 [0.95, 1.12]
2.3.1 Trials with low risk of bias	7	37932	Risk Ratio (M-H, Random, 95% CI)	1.03 [0.95, 1.12]

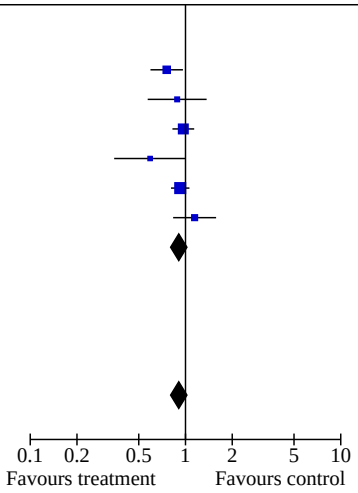
Analysis 2.1. Comparison 2: Homocysteine-lowering treatment versus placebo or standard care (Sensitivity analysis), Outcome 1: Myocardial infarction

Study or Subgroup	Treatment Events	Treatment Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
2.1.1 Trials with low risk of bias						
HOPE-2 2006	341	2758	349	2764	28.5%	0.98 [0.85 , 1.13]
NORVIT 2006	534	2806	163	943	22.0%	1.10 [0.94 , 1.29]
SEARCH 2010	431	6033	429	6031	33.5%	1.00 [0.88 , 1.14]
SU.FOL.OM3 2010	28	1242	32	1259	2.2%	0.89 [0.54 , 1.46]
VITATOPS 2010	118	4089	114	4075	8.6%	1.03 [0.80 , 1.33]
WAFACS 2008	65	2721	74	2721	5.1%	0.88 [0.63 , 1.22]
Subtotal (95% CI)		**19649**		**17793**	**100.0%**	**1.01 [0.94 , 1.09]**
Total events:	1517		1161			
Heterogeneity: Tau² = 0.00; Chi² = 2.30, df = 5 (P = 0.81); I² = 0%						
Test for overall effect: Z = 0.26 (P = 0.79)						
Total (95% CI)		**19649**		**17793**	**100.0%**	**1.01 [0.94 , 1.09]**
Total events:	1517		1161			
Heterogeneity: Tau² = 0.00; Chi² = 2.30, df = 5 (P = 0.81); I² = 0%						
Test for overall effect: Z = 0.26 (P = 0.79)						
Test for subgroup differences: Not applicable						



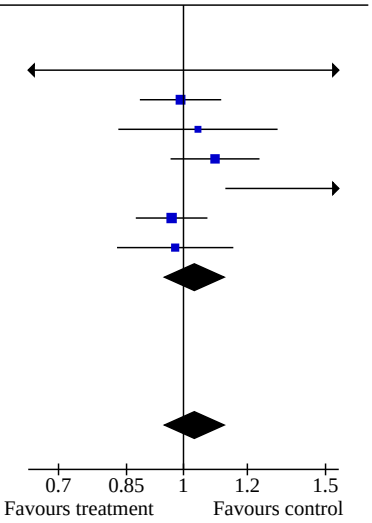
Analysis 2.2. Comparison 2: Homocysteine-lowering treatment versus placebo or standard care (Sensitivity analysis), Outcome 2: Stroke

Study or Subgroup	Treatment Events	Treatment Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
2.2.1 Trials with low risk of bias						
HOPE-2 2006	111	2758	147	2764	17.2%	0.76 [0.59 , 0.96]
NORVIT 2006	71	2806	27	943	6.7%	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	4.7%	0.59 [0.35 , 1.01]
VITATOPS 2010	360	4089	388	4075	32.2%	0.92 [0.81 , 1.06]
WAFACS 2008	79	2721	69	2721	11.4%	1.14 [0.83 , 1.57]
Subtotal (95% CI)		**19649**		**17793**	**100.0%**	**0.90 [0.80 , 1.02]**
Total events:	919		953			
Heterogeneity: Tau² = 0.01; Chi² = 7.39, df = 5 (P = 0.19); I² = 32%						
Test for overall effect: Z = 1.62 (P = 0.10)						
Total (95% CI)		**19649**		**17793**	**100.0%**	**0.90 [0.80 , 1.02]**
Total events:	919		953			
Heterogeneity: Tau² = 0.01; Chi² = 7.39, df = 5 (P = 0.19); I² = 32%						
Test for overall effect: Z = 1.62 (P = 0.10)						
Test for subgroup differences: Not applicable						



Analysis 2.3. Comparison 2: Homocysteine-lowering treatment versus placebo or standard care (Sensitivity analysis), Outcome 3: Death from any cause

Study or Subgroup	Treatment Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
2.3.1 Trials with low risk of bias						
BVAIT 2009	0	248	2	242	0.1%	0.20 [0.01 , 4.04]
HOPE-2 2006	470	2758	475	2764	22.8%	0.99 [0.88 , 1.11]
NORVIT 2006	276	2806	89	943	10.3%	1.04 [0.83 , 1.31]
SEARCH 2010	463	6033	423	6031	21.0%	1.09 [0.96 , 1.24]
SU.FOL.OM3 2010	72	1242	45	1259	4.8%	1.62 [1.13 , 2.33]
VITATOPS 2010	614	4089	633	4075	25.2%	0.97 [0.87 , 1.07]
WAFACS 2008	250	2721	256	2721	15.8%	0.98 [0.83 , 1.15]
Subtotal (95% CI)		**19897**		**18035**	**100.0%**	**1.03 [0.95 , 1.12]**
Total events:	2145		1923			
Heterogeneity: Tau² = 0.00; Chi² = 10.14, df = 6 (P = 0.12); I² = 41%						
Test for overall effect: Z = 0.71 (P = 0.48)						
Total (95% CI)		**19897**		**18035**	**100.0%**	**1.03 [0.95 , 1.12]**
Total events:	2145		1923			
Heterogeneity: Tau² = 0.00; Chi² = 10.14, df = 6 (P = 0.12); I² = 41%						
Test for overall effect: Z = 0.71 (P = 0.48)						
Test for subgroup differences: Not applicable						

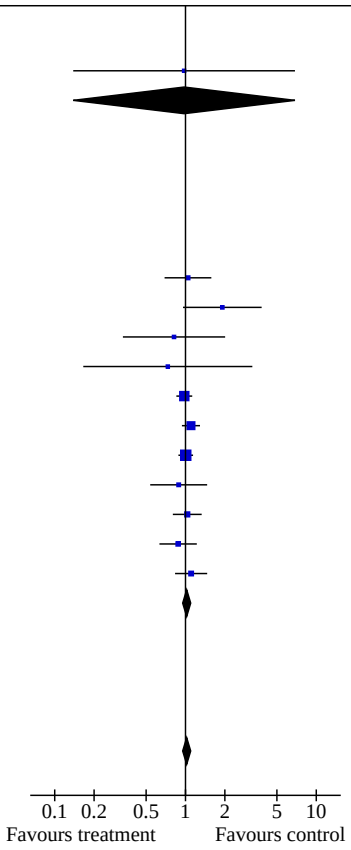


Comparison 3. Homocysteine-lowering treatment versus placebo (Subgoup analysis)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
3.1 Myocardial Infarction	12	46699	Risk Ratio (M-H, Random, 95% CI)	1.02 [0.95, 1.10]
3.1.1 Without history of cardiovascular disease	1	490	Risk Ratio (M-H, Random, 95% CI)	0.98 [0.14, 6.87]
3.1.2 With history of cardiovascular disease	11	46209	Risk Ratio (M-H, Random, 95% CI)	1.02 [0.95, 1.10]
3.2 Stroke	10	44224	Risk Ratio (M-H, Random, 95% CI)	0.90 [0.82, 0.99]
3.2.1 Without history of cardiovascular disease	1	490	Risk Ratio (M-H, Random, 95% CI)	0.20 [0.01, 4.04]
3.2.2 With history of cardiovascular disease	9	43734	Risk Ratio (M-H, Random, 95% CI)	0.90 [0.82, 0.99]
3.3 Death	11	44817	Risk Ratio (M-H, Random, 95% CI)	1.01 [0.96, 1.06]
3.3.1 Without history of cardiovascular disease	1	490	Risk Ratio (M-H, Random, 95% CI)	0.20 [0.01, 4.04]
3.3.2 With history of cardiovascular disease	10	44327	Risk Ratio (M-H, Random, 95% CI)	1.01 [0.96, 1.06]

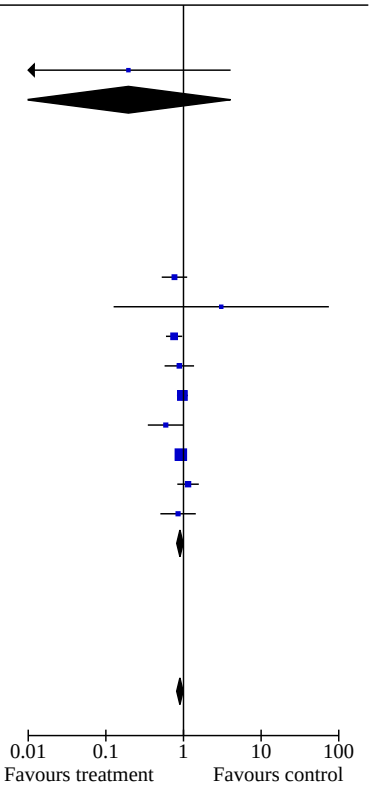
Analysis 3.1. Comparison 3: Homocysteine-lowering treatment versus placebo (Subgoup analysis), Outcome 1: Myocardial Infarction

Study or Subgroup	Treatment Events	Treatment Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
3.1.1 Without history of cardiovascular disease						
BVAIT 2009	2	248	2	242	0.1%	0.98 [0.14 , 6.87]
Subtotal (95% CI)		**248**		**242**	**0.1%**	**0.98 [0.14 , 6.87]**
Total events:	2		2			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.02 (P = 0.98)						
3.1.2 With history of cardiovascular disease						
B-PROOF 2015	45	1461	43	1458	2.9%	1.04 [0.69 , 1.58]
CHAOS 2002	23	942	12	940	1.0%	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	25.4%	0.98 [0.85 , 1.13]
NORVIT 2006	534	2806	163	943	19.5%	1.10 [0.94 , 1.29]
SEARCH 2010	431	6033	429	6031	29.8%	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	7.7%	1.03 [0.80 , 1.33]
WAFACS 2008	65	2721	74	2721	4.6%	0.88 [0.63 , 1.22]
WENBIT 2008	190	2311	58	779	6.2%	1.10 [0.83 , 1.46]
Subtotal (95% CI)		**24803**		**21406**	**99.9%**	**1.02 [0.95 , 1.10]**
Total events:	1786		1288			
Heterogeneity: Tau² = 0.00; Chi² = 6.28, df = 10 (P = 0.79); I² = 0%						
Test for overall effect: Z = 0.59 (P = 0.56)						
Total (95% CI)		**25051**		**21648**	**100.0%**	**1.02 [0.95 , 1.10]**
Total events:	1788		1290			
Heterogeneity: Tau² = 0.00; Chi² = 6.28, df = 11 (P = 0.85); I² = 0%						
Test for overall effect: Z = 0.58 (P = 0.56)						
Test for subgroup differences: Chi² = 0.00, df = 1 (P = 0.96), I² = 0%						



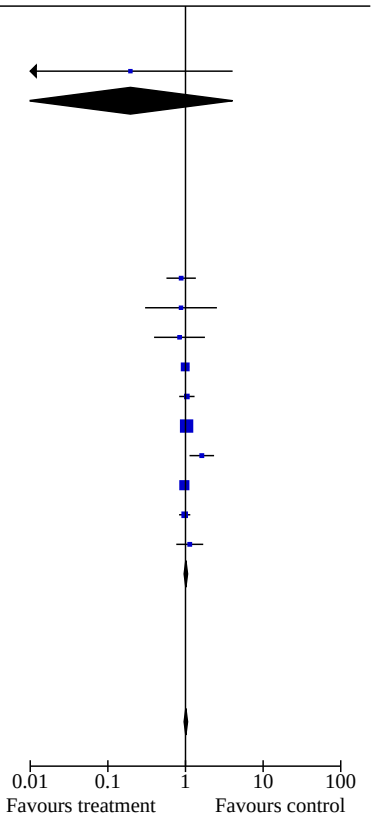
Analysis 3.2. Comparison 3: Homocysteine-lowering treatment versus placebo (Subgoup analysis), Outcome 2: Stroke

Study or Subgroup	Treatment Events	Treatment Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
3.2.1 Without history of cardiovascular disease						
BVAIT 2009	0	248	2	242	0.1%	0.20 [0.01 , 4.04]
Subtotal (95% CI)		**248**		**242**	**0.1%**	**0.20 [0.01 , 4.04]**
Total events:	0		2			
Heterogeneity: Not applicable						
Test for overall effect: Z = 1.06 (P = 0.29)						
3.2.2 With history of cardiovascular disease						
B-PROOF 2015	46	1461	60	1458	6.0%	0.77 [0.52 , 1.12]
FOLARDA 2004	1	140	0	143	0.1%	3.06 [0.13 , 74.58]
HOPE-2 2006	111	2758	147	2764	13.8%	0.76 [0.59 , 0.96]
NORVIT 2006	71	2806	27	943	4.5%	0.88 [0.57 , 1.37]
SEARCH 2010	277	6033	286	6031	26.7%	0.97 [0.82 , 1.14]
SU.FOL.OM3 2010	21	1242	36	1259	3.1%	0.59 [0.35 , 1.01]
VITATOPS 2010	360	4089	388	4075	34.2%	0.92 [0.81 , 1.06]
WAFACS 2008	79	2721	69	2721	8.3%	1.14 [0.83 , 1.57]
WENBIT 2008	48	2311	19	779	3.2%	0.85 [0.50 , 1.44]
Subtotal (95% CI)		**23561**		**20173**	**99.9%**	**0.90 [0.82 , 0.99]**
Total events:	1014		1032			
Heterogeneity: Tau² = 0.00; Chi² = 8.82, df = 8 (P = 0.36); I² = 9%						
Test for overall effect: Z = 2.16 (P = 0.03)						
Total (95% CI)		**23809**		**20415**	**100.0%**	**0.90 [0.82 , 0.99]**
Total events:	1014		1034			
Heterogeneity: Tau² = 0.00; Chi² = 9.81, df = 9 (P = 0.37); I² = 8%						
Test for overall effect: Z = 2.19 (P = 0.03)						
Test for subgroup differences: Chi² = 0.98, df = 1 (P = 0.32), I² = 0%						



Analysis 3.3. Comparison 3: Homocysteine-lowering treatment versus placebo (Subgoup analysis), Outcome 3: Death

Study or Subgroup	Treatment Events	Treatment Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
3.3.1 Without history of cardiovascular disease						
BVAIT 2009	0	248	2	242	0.0%	0.20 [0.01 , 4.04]
Subtotal (95% CI)		**248**		**242**	**0.0%**	**0.20 [0.01 , 4.04]**
Total events:	0		2			
Heterogeneity: Not applicable						
Test for overall effect: Z = 1.06 (P = 0.29)						
3.3.2 With history of cardiovascular disease						
B-PROOF 2015	37	1461	42	1458	1.3%	0.88 [0.57 , 1.36]
FOLARDA 2004	6	140	7	143	0.2%	0.88 [0.30 , 2.54]
GOES 2003	12	300	14	293	0.4%	0.84 [0.39 , 1.78]
HOPE-2 2006	470	2758	475	2764	18.7%	0.99 [0.88 , 1.11]
NORVIT 2006	276	2806	89	943	4.9%	1.04 [0.83 , 1.31]
SEARCH 2010	983	6033	951	6031	37.7%	1.03 [0.95 , 1.12]
SU.FOL.OM3 2010	72	1242	45	1259	1.9%	1.62 [1.13 , 2.33]
VITATOPS 2010	614	4089	633	4075	24.1%	0.97 [0.87 , 1.07]
WAFACS 2008	250	2721	256	2721	9.1%	0.98 [0.83 , 1.15]
WENBIT 2008	101	2311	30	779	1.6%	1.13 [0.76 , 1.69]
Subtotal (95% CI)		**23861**		**20466**	**100.0%**	**1.01 [0.96 , 1.06]**
Total events:	2821		2542			
Heterogeneity: Tau² = 0.00; Chi² = 8.86, df = 9 (P = 0.45); I² = 0%						
Test for overall effect: Z = 0.42 (P = 0.67)						
Total (95% CI)		**24109**		**20708**	**100.0%**	**1.01 [0.96 , 1.06]**
Total events:	2821		2544			
Heterogeneity: Tau² = 0.00; Chi² = 9.99, df = 10 (P = 0.44); I² = 0%						
Test for overall effect: Z = 0.41 (P = 0.68)						
Test for subgroup differences: Chi² = 1.13, df = 1 (P = 0.29), I² = 11.6%						

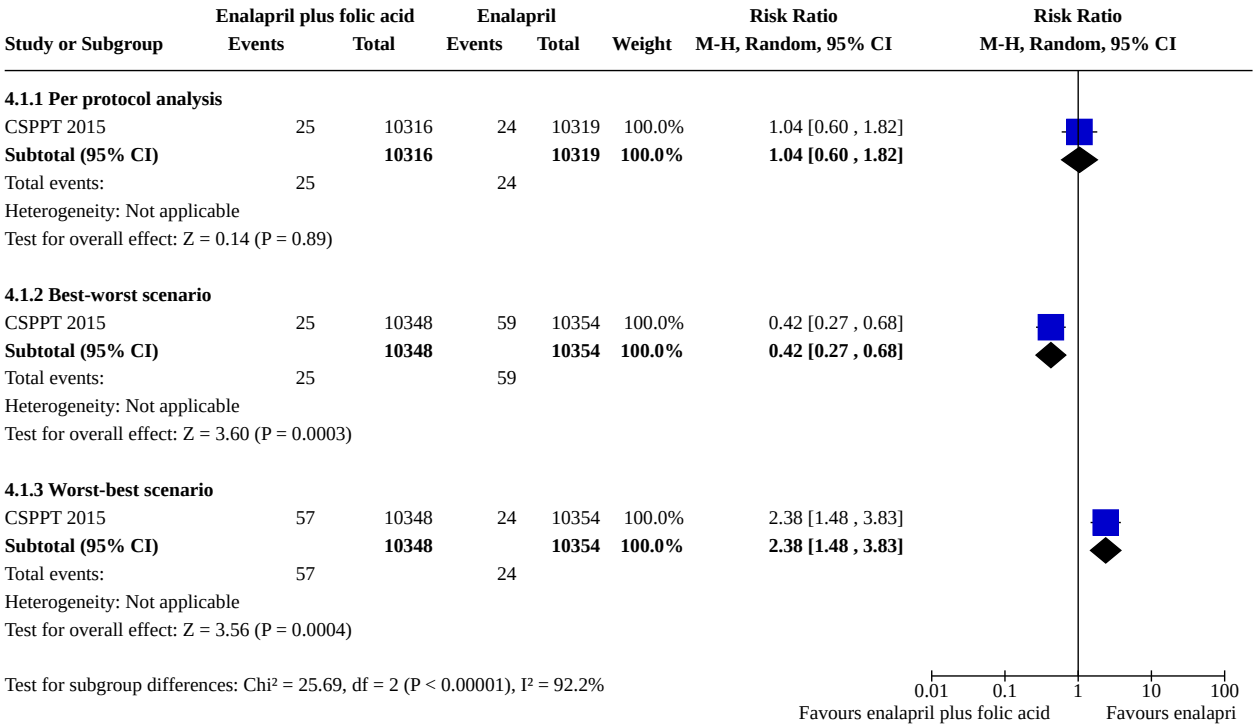


Comparison 4. Homocysteine-lowering treatment (folic acid) plus antihypertensive therapy (enalapril) versus antihypertensive therapy (enalapril) (Sensitivity analysis)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
4.1 Myocardial infarction	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.1.1 Per protocol analysis	1	20635	Risk Ratio (M-H, Random, 95% CI)	1.04 [0.60, 1.82]
4.1.2 Best-worst scenario	1	20702	Risk Ratio (M-H, Random, 95% CI)	0.42 [0.27, 0.68]
4.1.3 Worst-best scenario	1	20702	Risk Ratio (M-H, Random, 95% CI)	2.38 [1.48, 3.83]
4.2 Stroke	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.2.1 Per protocol analysis	1	20635	Risk Ratio (M-H, Random, 95% CI)	0.79 [0.68, 0.93]
4.2.2 Best-worst scenario	1	20702	Risk Ratio (M-H, Random, 95% CI)	0.72 [0.62, 0.84]
4.2.3 Worst-best scenario	1	20702	Risk Ratio (M-H, Random, 95% CI)	0.88 [0.76, 1.03]

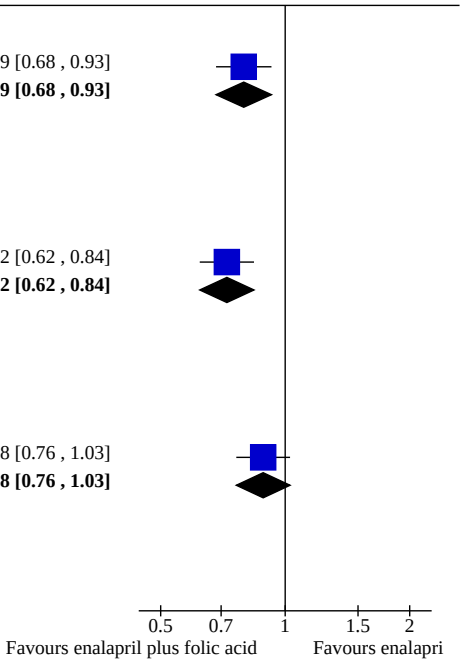
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
4.3 Death from any cause	1		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.3.1 Per protocol analysis	1	20635	Risk Ratio (M-H, Random, 95% CI)	0.94 [0.81, 1.10]
4.3.2 Best-worst scenario	1	20702	Risk Ratio (M-H, Random, 95% CI)	0.85 [0.73, 0.99]
4.3.3 Worst-best scenario	1	20702	Risk Ratio (M-H, Random, 95% CI)	1.04 [0.90, 1.21]

Analysis 4.1. Comparison 4: Homocysteine-lowering treatment (folic acid) plus antihypertensive therapy (enalapril) versus antihypertensive therapy (enalapril) (Sensitivity analysis), Outcome 1: Myocardial infarction



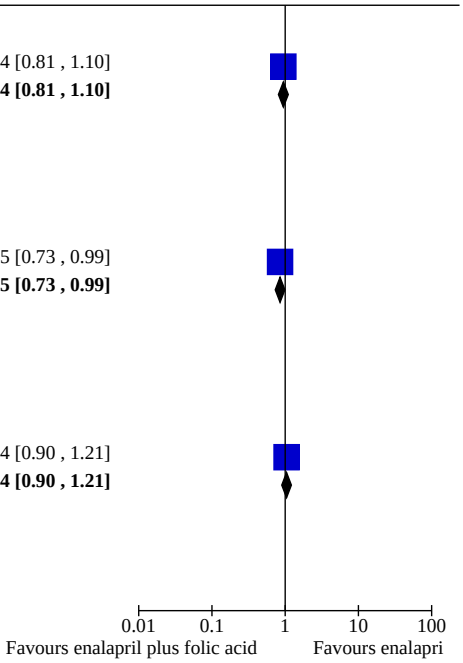
Analysis 4.2. Comparison 4: Homocysteine-lowering treatment (folic acid) plus antihypertensive therapy (enalapril) versus antihypertensive therapy (enalapril) (Sensitivity analysis), Outcome 2: Stroke

Study or Subgroup	Enalapril plus folic acid Events	Total	Enalapril Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
4.2.1 Per protocol analysis						
CSPPT 2015	281	10316	354	10319	100.0%	0.79 [0.68 , 0.93]
Subtotal (95% CI)		**10316**		**10319**	**100.0%**	**0.79 [0.68 , 0.93]**
Total events:	281		354			
Heterogeneity: Not applicable						
Test for overall effect: Z = 2.93 (P = 0.003)						
4.2.2 Best-worst scenario						
CSPPT 2015	281	10348	389	10354	100.0%	0.72 [0.62 , 0.84]
Subtotal (95% CI)		**10348**		**10354**	**100.0%**	**0.72 [0.62 , 0.84]**
Total events:	281		389			
Heterogeneity: Not applicable						
Test for overall effect: Z = 4.21 (P < 0.0001)						
4.2.3 Worst-best scenario						
CSPPT 2015	313	10348	354	10354	100.0%	0.88 [0.76 , 1.03]
Subtotal (95% CI)		**10348**		**10354**	**100.0%**	**0.88 [0.76 , 1.03]**
Total events:	313		354			
Heterogeneity: Not applicable						
Test for overall effect: Z = 1.61 (P = 0.11)						
Test for subgroup differences: Chi² = 3.48, df = 2 (P = 0.18), I² = 42.5%						



Analysis 4.3. Comparison 4: Homocysteine-lowering treatment (folic acid) plus antihypertensive therapy (enalapril) versus antihypertensive therapy (enalapril) (Sensitivity analysis), Outcome 3: Death from any cause

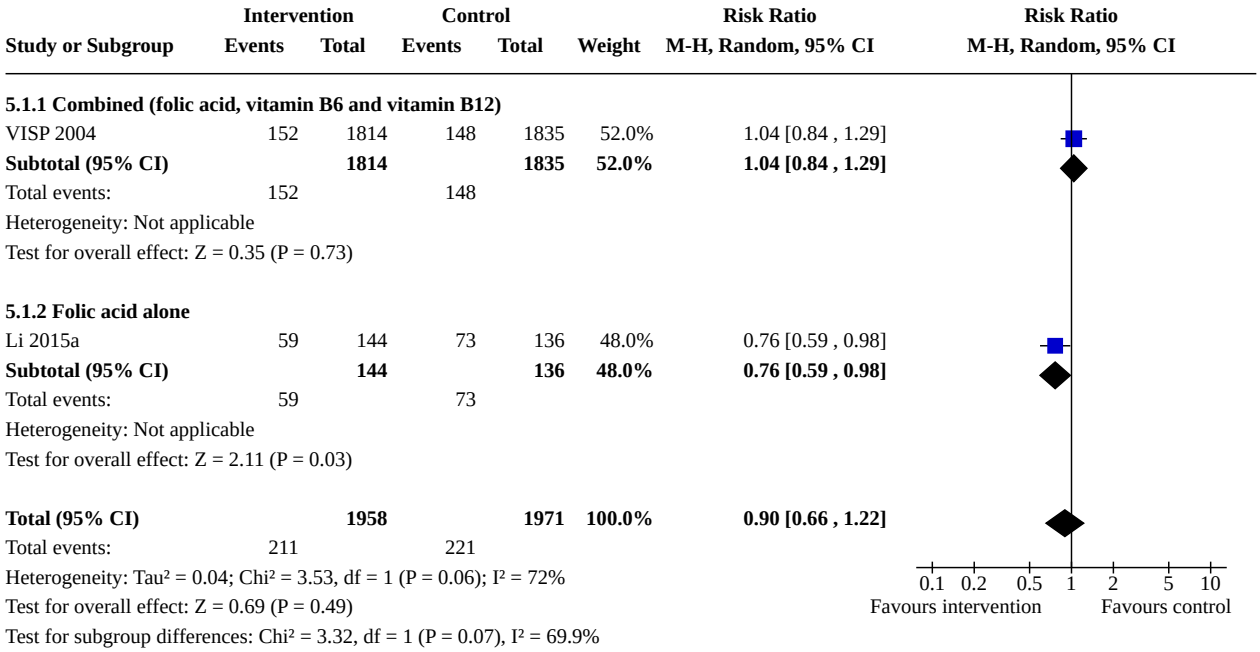
Study or Subgroup	Enalapril plus folic acid Events	Total	Enalapril Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
4.3.1 Per protocol analysis						
CSPPT 2015	302	10316	320	10319	100.0%	0.94 [0.81 , 1.10]
Subtotal (95% CI)		**10316**		**10319**	**100.0%**	**0.94 [0.81 , 1.10]**
Total events:	302		320			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.73 (P = 0.47)						
4.3.2 Best-worst scenario						
CSPPT 2015	302	10348	355	10354	100.0%	0.85 [0.73 , 0.99]
Subtotal (95% CI)		**10348**		**10354**	**100.0%**	**0.85 [0.73 , 0.99]**
Total events:	302		355			
Heterogeneity: Not applicable						
Test for overall effect: Z = 2.09 (P = 0.04)						
4.3.3 Worst-best scenario						
CSPPT 2015	334	10348	320	10354	100.0%	1.04 [0.90 , 1.21]
Subtotal (95% CI)		**10348**		**10354**	**100.0%**	**1.04 [0.90 , 1.21]**
Total events:	334		320			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.56 (P = 0.57)						
Test for subgroup differences: Chi² = 3.53, df = 2 (P = 0.17), I² = 43.3%						



Comparison 5. Homocysteine-lowering treatment at high dose versus low dose (Subgoup analysis)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
5.1 Stroke	2	3929	Risk Ratio (M-H, Random, 95% CI)	0.90 [0.66, 1.22]
5.1.1 Combined (folic acid, vitamin B6 and vitamin B12)	1	3649	Risk Ratio (M-H, Random, 95% CI)	1.04 [0.84, 1.29]
5.1.2 Folic acid alone	1	280	Risk Ratio (M-H, Random, 95% CI)	0.76 [0.59, 0.98]

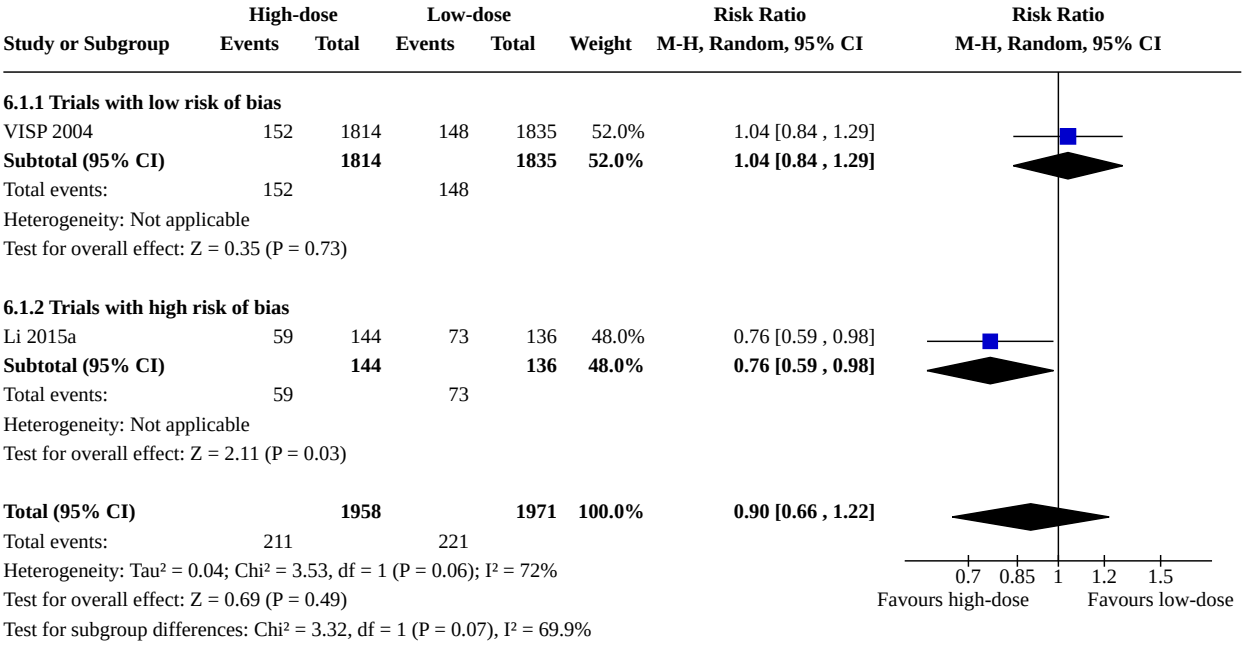
Analysis 5.1. Comparison 5: Homocysteine-lowering treatment at high dose versus low dose (Subgoup analysis), Outcome 1: Stroke



Comparison 6. Homocysteine-lowering treatment (high dose) versus Homocysteine-lowering treatment (low dose) (Sensitivity analysis)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
6.1 Stroke	2	3929	Risk Ratio (M-H, Random, 95% CI)	0.90 [0.66, 1.22]
6.1.1 Trials with low risk of bias	1	3649	Risk Ratio (M-H, Random, 95% CI)	1.04 [0.84, 1.29]
6.1.2 Trials with high risk of bias	1	280	Risk Ratio (M-H, Random, 95% CI)	0.76 [0.59, 0.98]

Analysis 6.1. Comparison 6: Homocysteine-lowering treatment (high dose) versus Homocysteine-lowering treatment (low dose) (Sensitivity analysis), Outcome 1: Stroke



APPENDICES

Appendix 1. 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)

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

MEDLINE

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 2. 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=(hyperhomocystinemi*)
#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 3. 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=(hyperhomocystinemi*)
#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 2017

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 OVID

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 (2014* or 2015* or 2016*).ed.

43 41 and 42

Embase OVID

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 (2014* or 2015* or 2016*).dd.

50 48 and 49

Web of Science

#26 #25 AND #24

#25 TS=((random* or blind* or allocat* or assign* or trial* or placebo* or crossover* or cross-over*))

#24 #23 AND #10

#23 #22 OR #21 OR #20 OR #19 OR #18 OR #17 OR #16 OR #15 OR #14 OR #13 OR #12 OR #11

#22 TS=(hyperhomocystein$emi*)

#21 TS=(hyperhomocystin$emi*)

#20 TS=(cardiovascular near/2 (disease* or event*))

#19 TS=(apoplexy)

#18 TS=((brain near/3 infarct*))

#17 TS=((cerebr* near/3 (accident* or infarct*)))

#16 TS=((stroke or strokes))

#15 TS=(heart attack*)

#14 TS=(heart infarct*)

#13 TS=(myocardial infarct*)

#12 TS=(angina)

#11 TS=((coronary near/3 disease*))

#10 #9 OR #8 OR #7 OR #6 OR #5 OR #4 OR #3 OR #2 OR #1

#9 TS=(pyridoxol*)

#8 TS=(cyanocobalamin*)

#7 TS=(cobalamin*)

#6 TS=(pyridoxin*)

#5 TS=((homocystin*) near/3 (low or reduc*))

#4 TS=((homocystein*) near/3 (low$ or reduc*))

#3 TS=(folate*)

#2 TS=("folic acid")

#1 TS=("vitamin b")

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 SIMPLECIEGO OR Pt ESTUDIO MULTICÉNTRICO) or ((tw ensaio or tw ensayo or tw trial) and (tw azar or tw acaso or tw placebo or tw control$ or tw aleat$ or tw random$ or (tw duplo and tw cego) or

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

Appendix 5. Domains for assessing of risk of bias in included studies

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.

Overall risk of bias

We considered studies to have an overall low risk of bias if they did not have high risk of bias in any of six individual domains (random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data or selective reporting), and if a definitive risk of bias assessment could be made for the majority (at least five of six) of domains. We did not include 'Other bias' in our overall assessment.

Appendix 6. Definitions of myocardial infarction (MI), stroke, unstable angina and death

Trial	Myocardial infarction	Stroke	Angina pectoris	Death	
B-PROOF 2015	Not available	Not available	Not available	Not available	
BVAIT 2009	Not available	Not available	Not available		
CSPPT 2015	Criteria for ischaemic symptoms or corresponding electrocardiographic changes plus evidence of myocardial damage.	Medical records and imaging data	Not measured	Evidence for death included death certificates from hospitals or reports of home visit by investigators	
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 postmortem evidence of another cause, deaths from myocardial infarction or stroke within 7 days after the event, deaths associated with cardiovascular interventions within 30 days after cardiovascular surgery or within 7 days after percutaneous interventions, and deaths from congestive heart failure, arrhythmia, pulmonary embolism or ruptured aortic aneurysm. Deaths from uncertain causes were presumed to be due to cardiovascular causes	Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined - a consensus document of the joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol 2000;36:959-69. [Erratum, J Am Coll Cardiol 2001;37:973.]: source not available

(Continued)

Li 2015a	Not measured	Not available	Not measured	Not measured	-
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 characteristic electrocardiographic evidence of myocardial infarction provided there was angiographic evidence of coronary artery disease		
VISP 2004	New ECG changes including Q waves	Evidence of sudden onset of focal neurologic deficit	Not available	Not available	

(Continued)

	or marked ST-T changes plus abnormal cardiac enzymes, cardiac symptoms plus abnormal enzymes or symptoms plus hyperacute ECG changes resolving with thrombolysis	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		
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
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

Date	Event	Description
21 September 2021	Review declared as stable	This review topic is considered not to be a priority for the current scope of the Heart Group.

HISTORY

Protocol first published: Issue 3, 2007
Review first published: Issue 4, 2009

Date	Event	Description
1 June 2017	New citation required and conclusions have changed	There is new information on stroke.
1 June 2017	New search has been performed	We updated the searches to June 2017. We found three new trials. This updated Cochrane Review now has four authors.
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. This updated Cochrane Review now has only three authors.
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 review.
Ivan Solà identified trials, extracted data, edited the 'Summary of findings' tables and drafted the review.
Dimitris Lathyris extracted and checked the data and reviewed the review.
Mark Dayer critically reviewed and amended the manuscript.

DECLARATIONS OF INTEREST

Arturo Marti-Carvajal: 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 Cochrane 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 Cochrane or any Cochrane review.
Ivan Solà: none known.
Dimitris Lathyris: none known.
Mark Dayer: none known.

SOURCES OF SUPPORT

Internal sources

- No sources of support provided

External sources

- Iberoamerican Cochrane Centre, Spain

  Academic
- Cochrane Heart Group, UK

  Academic

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

1. Number needed to treat for an additional beneficial outcome if the risk reduction was significant (P value = < 0.05)
2. Harbord and Peters tests for estimation publication bias.
3. Bayes factors
4. Fragility Indices
5. Trials including participants without cardiovascular disease versus trials including participants with cardiovascular disease (considered post-hoc).
6. We considered studies to have an overall low risk of bias if they did not have high risk of bias in any of six individual domains (random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data or selective reporting), and if a definitive 'Risk of bias' assessment could be made for the majority (at least five of six) of domains. We did not include 'Other bias' in our overall assessment.

Second update (Martí-Carvajal 2015): included trial sequential analyses.

First update (Martí-Carvajal 2013): In the first version of the review (Martí-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 review has been updated at each step to current recommendations of Cochrane, including updates to the Plain Language Summary and inclusion of the quality of the evidence assessed according to GRADE ('Summary of findings').

INDEX TERMS

Medical Subject Headings (MeSH)

Angina Pectoris [prevention & control]; Cardiovascular Diseases [etiology] [*prevention & control]; Cause of Death; Folic Acid [therapeutic use]; Hyperhomocysteinemia [complications] [*therapy]; Myocardial Infarction [epidemiology] [prevention & control]; Randomized Controlled Trials as Topic; Risk Factors; Stroke [epidemiology] [prevention & control]; Vitamin B 12 [therapeutic use]; Vitamin B 6 [therapeutic use]; Vitamin B Complex [*therapeutic use]

MeSH check words

Humans