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Interventions for lowering plasma homocysteine levels in dialysis patients

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ABSTRACT

Background

People with end-stage kidney disease (ESKD) have high rates of cardiovascular events. Randomised controlled trials (RCTs) of homocysteine-lowering therapies have not shown reductions in cardiovascular event rates in the general population. However, people with kidney disease have higher levels of homocysteine and may have different mechanisms of cardiovascular disease. We performed a systematic review of the effect of homocysteine-lowering therapies in people with ESKD.

Objectives

To evaluate the benefits and harms of established homocysteine lowering therapy (folic acid, vitamin B6, vitamin B12) on all-cause mortality and cardiovascular event rates in patients with ESKD.

Search methods

We searched Cochrane Kidney and Transplant’s Specialised Register to 25 January 2016 through contact with the Information Specialist using search terms relevant to this review.

Selection criteria

Studies conducted in people with ESKD that reported at least 100 patient-years of follow-up and assessed the effect of therapies that are known to have homocysteine-lowering properties were included.
Data collection and analysis

Two authors independently extracted data using a standardised form. The primary outcome was cardiovascular mortality. Secondary outcomes included all-cause mortality, incident cardiovascular disease (fatal and nonfatal myocardial infarction and coronary revascularisation), cerebrovascular disease (stroke and cerebrovascular revascularisation), peripheral vascular disease (lower limb amputation), venous thromboembolic disease (deep vein thrombosis and pulmonary embolism), thrombosis of dialysis access, and adverse events. The effects of homocysteine-lowering therapies on outcomes were assessed with meta-analyses using random-effects models. Prespecified subgroup and sensitivity analyses were conducted.

Main results

We included six studies that reported data on 2452 participants with ESKD. Interventions investigated were folic acid with or without other vitamins (vitamin B₆, vitamin B₁₂). Participants’ mean age was 48 to 65 years, and proportions of male participants ranged from 50% to 98%.

Homocysteine-lowering therapy probably leads to little or no effect on cardiovascular mortality (4 studies, 1186 participants: RR 0.93, 95% CI 0.70 to 1.22). There was no evidence of heterogeneity among the included studies ($I^2 = 0\%$). Homocysteine-lowering therapy had little or no effect on all-cause mortality or any other of this review’s secondary outcomes. All prespecified subgroup and sensitivity analyses demonstrated little or no difference. Reported adverse events were mild and there was no increase in the incidence of adverse events from homocysteine-lowering therapies (3 studies, 1248 participants: RR 1.12, 95% CI 0.51 to 2.47; $I^2 = 0\%$). Overall, studies were assessed as being at low risk of bias and there was no evidence of publication bias.

Authors’ conclusions

Homocysteine-lowering therapies were not found to reduce mortality (cardiovascular and all-cause) or cardiovascular events among people with ESKD.

Plain language summary

Interventions for lowering plasma homocysteine levels in dialysis patients

Background

People with advanced kidney disease frequently develop heart disease, which is the most common cause of deaths in these people. An increased level of the amino acid (homocysteine) in the blood is a risk factor for heart disease in people with advanced kidney disease. Therapies that reduce homocysteine levels (e.g. folic acid, vitamins B₆ and B₁₂) are often used, but the benefits and harms of their use are unclear. We aimed to assess the benefits and harms of homocysteine-lowering therapies in people with advanced kidney disease who were on dialysis.

Study characteristics

From a search of the literature in January 2016, we identified six randomised controlled trials that involved 2452 participants aged between 48 and 65 years to be analysed.

Key results

We found that homocysteine-lowering therapies had no benefits for heart health in people with advanced kidney disease who were on dialysis. These therapies did not achieve any reduction in rates of heart disease-related death. However, homocysteine-lowering therapies were generally well tolerated, and had a mild side effect profile.

Quality of the evidence

Overall, studies were assessed as high quality.
BACKGROUND

Description of the condition

Dialysis-dependent end-stage kidney disease (ESKD) patients have reduced life expectancy, with annual mortality of 14.8% in Australia (McDonald 2007), and 16.7% in the USA (USRDS 2007). Chronic kidney disease (CKD) is an independent and powerful risk factor for cardiovascular disease (Go 2004; Weiner 2004). Cardiovascular mortality is significant in CKD (de Jager 2009). For dialysis-dependent ESKD patients, death from cardiovascular disease is 10 to 100 times higher than in age and sex-matched controls (Foley 1998).

The increased prevalence of cardiovascular disease among ESKD patients is not completely accounted for by the presence of traditional risk factors such as hypertension, dyslipidaemia, diabetes mellitus, smoking, and left ventricular hypertrophy (Baigent 2000). Thus, there has been increasing investigation of nontraditional risk factors such as anaemia, hyperparathyroidism and hyperhomocysteinaemia.

Description of the intervention

Homocysteine is an amino acid produced from the metabolism of methionine. It is believed to play a role in the pathogenesis of atherosclerosis by damaging the endothelium and promoting clotting (Eikelboom 1999). Epidemiological studies have shown homocysteine to be an independent risk factor for cardiovascular disease (HSC 2002). Elevated homocysteine level is a predictor of vascular disease including stroke, myocardial infarction (MI, heart attack), atherosclerosis (thickening of artery walls), arterial and venous thrombosis (clotting) and cardiovascular death in the general population (HSC 2002; Wald 2002) and in people with ESKD (Chauveau 1993; Moustapha 1998; Robinson 1996). In contrast to the well-documented association between homocysteine levels and vascular events in the general population, the association between homocysteine levels and risk for atherothrombotic disease is not consistent among the ESKD population. Some investigators have described an inverse association between homocysteine levels and clinical outcomes in the ESKD patients with low levels of homocysteine being associated with worse outcomes (Kalantar-Zadeh 2004; Wrone 2001). However, even a mild increase in the homocysteine level appears to be a vascular risk factor in the general population and there is high prevalence of hyperhomocysteinaemia in ESKD. Thus, the paradoxical reverse association between homocysteine and clinical outcome in ESKD patients does not as such refute a possible role for homocysteine in the vascular pathogenesis. Furthermore, it has also been shown that association of hyperhomocysteinaemia and worse clinical outcomes persists in ESKD patients without chronic inflammation-malnutrition state (Ducloux 2006). These authors postulate that the burden of chronic inflammation-malnutrition might mask the true relationship between hyperhomocysteinaemia and cardiovascular risk among dialysis-dependent ESKD patients.

How the intervention might work

Because kidney metabolism is the primary means by which homocysteine is cleared, plasma homocysteine has a strong inverse correlation with estimated glomerular filtration rate (Freidman 2001). Among patients with ESKD, the prevalence of hyperhomocysteinaemia is 85% to 100% (Bostom 1999).

In classical homocystinuria, plasma total homocysteine concentrations are very high (100 to 400 μmol/L), and untreated patients die prematurely from venous thromboembolism and malignant arterial disease. Long-term treatment aimed at lowering homocysteine levels has been extremely effective in reducing the potentially life-threatening vascular risk in these patients modified by agents that lower homocysteine such as folic acid and vitamins B6 and B12. Of the homocysteine-lowering therapies, folic acid is the most effective and consistent in reducing homocysteine levels in ESKD (van Guldener 2006).

Why it is important to do this review

A limited number of studies of homocysteine-lowering therapy in ESKD patients have been conducted which individually have been unable to find a statistically significant effect on surrogate markers of cardiovascular disease, cardiovascular events or all-cause mortality (ASFAST 2004; HOST Study 2004; Vianna 2007; Wrone 2004). The picture is confused by the inability of studies to normalise homocysteine levels in most patients, despite the administration of larger doses of folic acid than in studies of other population groups (ASFAST 2004; Gonin 2005).

Although there is no RCT evidence to support folic acid or vitamin B interventions, multitreatment supplementation for patients with ESKD is commonly practiced. The K/DOQI Clinical Practice Guidelines for Cardiovascular Disease in Dialysis Patients promote the administration of folate, vitamins B6 and B12 to compensate for dialysate losses and prevent elevation of homocysteine on the grounds of cardiovascular risk (K/DOQI 2005). The group also acknowledged the lack of evidence in the area and state that further data are required regarding the effect of vitamin therapy on clinical outcomes. Furthermore potential harms associated with folic acid supplements have not been excluded (Zoccali 2010).

In light of the burden of cardiovascular disease for patients with ESKD, the effectiveness or otherwise of any potentially effective interventions needs to be clearly established. This review aimed to assess the benefits and harms of homocysteine lowering therapy among people with ESKD to guide decision making and improve outcomes for patients.
OBJECTIVES

To evaluate the benefits and harms of established homocysteine lowering therapy (folic acid, vitamin B₆, vitamin B₁₂) on all-cause mortality and cardiovascular event rates in patients with ESKD.

METHODS

Criteria for considering studies for this review

Types of studies

RCTs and quasi-RCTs (RCTs in which allocation to treatment was obtained by alteration, use of alternate medical records, date of birth or other predictable methods) looking at the use of established homocysteine lowering therapy, with a minimum of 100 patient years were included to reduce the risk of reporting or publication bias. Sequential and cross-over studies were excluded.

Types of participants

We included adults (aged over 18 years) with ESKD defined as those requiring maintenance dialysis. Patients with functioning kidney transplants were excluded.

Types of interventions

Studies randomising patients to therapies which have proven efficacy in lowering homocysteine levels were included. Studies of regimens in which the main mechanism of action is thought not to be homocysteine lowering were excluded (e.g. simvastatin plus folic acid, N-acetyl cysteine). We investigated the following comparisons.

- Homocysteine-lowering therapy versus placebo or usual care
- Higher dose homocysteine-lowering therapy versus lower dose homocysteine-lowering therapy
- Any schedule of treatment
- Any route of treatment.

Types of outcome measures

Primary outcomes

- Cardiovascular mortality

Secondary outcomes

- All-cause mortality
- Cardiovascular disease
  - Fatal and nonfatal MI
  - Coronary revascularisation
- Cerebrovascular disease
  - Stroke
  - Cerebrovascular revascularisation
- Peripheral vascular disease and venous thromboembolic disease
  - Lower limb amputation
  - Deep vein thrombosis (DVT) and pulmonary embolism (PE)
- Kidney-specific outcomes
  - Thrombosis of dialysis access
- Adverse events from folate-based therapy
  - Gastrointestinal events
  - Dermatological events
  - Neurological events
  - Malignancy incidence and mortality
  - Any self-reported adverse events.

Search methods for identification of studies

Electronic searches

We searched Cochrane Kidney and Transplant’s Specialised Register to 25 January 2016 through contact with the Information Specialist using search terms relevant to this review. The Specialised Register contains studies identified from the following sources.

1. Quarterly searches of the Cochrane Central Register of Controlled Trials (CENTRAL)
2. Weekly searches of MEDLINE OVID SP
3. Handsearching of kidney-related journals and the proceedings of major kidney conferences
4. Searching of the current year of EMBASE OVID SP
5. Weekly current awareness alerts for selected kidney journals

Studies contained in the Specialised Register are identified through search strategies for CENTRAL, MEDLINE, and EMBASE based on the scope of Cochrane Kidney and Transplant. Details of these strategies as well as a list of handsearched journals, conference proceedings and current awareness alerts are available in the Specialised Register section of information about the Cochrane Kidney and Transplant.

See Appendix 1 for search terms used in strategies for this review.
Searching other resources

1. Reference lists of review articles, relevant studies and clinical practice guidelines.
2. Letters seeking information about unpublished or incomplete studies to investigators known to be involved in previous studies.

Data collection and analysis

Steps in data collection and analyses are outlined below.

Selection of studies

The search strategy described was used to obtain titles and abstracts of studies that may be relevant to the review. Titles and abstracts were screened independently by two authors, and studies that were not applicable were discarded. However, studies and reviews that might include relevant data or information on studies were retained initially. Two authors independently assessed retrieved abstracts and the full text as necessary of these studies to determine which studies satisfy the inclusion criteria.

Data extraction and management

Data extraction was carried out independently by two authors using standard data extraction forms. Studies reported in non-English language journals were translated before assessment. Where more than one publication of one RCT existed, only the publication with the most complete data were to be included; however, we did not find any duplicate publications. Disagreements were resolved by consultation.

Assessment of risk of bias in included studies

The following items were independently assessed by two authors using the risk of bias assessment tool (Higgins 2011) (see Appendix 2).

- Was there adequate sequence generation (selection bias)?
- Was allocation adequately concealed (selection bias)?
- Was knowledge of the allocated interventions adequately prevented during the study?
  - Participants and personnel (performance bias)
  - Outcome assessors (detection bias)
- Were incomplete outcome data adequately addressed (attrition bias)?
- Are reports of the study free of suggestion of selective outcome reporting (reporting bias)?
- Was the study apparently free of other problems that could put it at a risk of bias?

Measures of treatment effect

For dichotomous outcomes (all-cause mortality, MI, coronary revascularisation, cardiovascular death, stroke, cerebrovascular revascularisation, lower limb amputation, thrombosis of arteriovenous (AV) access, DVT, PE), results were expressed as risk ratio (RR) with 95% confidence intervals (CI). Data were pooled using the random-effects model but the fixed-effect model was also analysed to ensure robustness of the model chosen and susceptibility to outliers.

Dealing with missing data

Any further information required from the original author was to be requested by written correspondence and any relevant information obtained in this manner was included in the review. Attrition rates, such as drop-outs, losses to follow-up and withdrawals were to be investigated. Issues of missing data and imputation methods (e.g. last-observation-carried-forward) were to be critically appraised (Higgins 2011).

Assessment of heterogeneity

Heterogeneity was analysed using a Chi² test on N-1 degrees of freedom, with an alpha of 0.05 used for statistical significance and with the I² test (Higgins 2003). I² values of 25%, 50% and 75% correspond to low, medium and high levels of heterogeneity.

Assessment of reporting biases

Publication bias was assessed using a funnel plot (Egger 1997). Attrition bias was assessed using the loss/event ratio.

Data synthesis

Data were pooled using the random-effects model but the fixed-effect model was also analysed to ensure robustness of the model chosen and susceptibility to outliers.

Subgroup analysis and investigation of heterogeneity

Subgroup analyses were conducted to explore possible sources of heterogeneity using inverse variance according to the following characteristics.

- Study intervention
- Exposure to folic acid among control group participants
- Proportion of participants with diabetes mellitus
- Proportion of participants with cardiac disease
- Study duration follow-up
- Study event number.

We aimed to analyse the ability of interventions to normalise homocysteine levels in a subgroup of studies that enrolled patients with elevated homocysteine levels. Plausible explanations for variations in treatment effect were explored using subgroup analyses based on study quality and length of follow-up.
Sensitivity analysis
Sensitivity analyses were conducted to ensure conclusions are robust to decisions made during the review process such as inclusion criteria and imputing of missing data. Where outcomes sought were reported in insufficient detail to allow meta-analysis and further information was not forthcoming from trialists, we planned to summarise these outcomes and assess with descriptive techniques. We planned to calculate the number of persons needed to treat to avoid one cardiovascular death if adequate data were available; however, our non-significant results did not permit making this calculation.

RESULTS

Description of studies
Detailed description of study search results and description of studies is outlined below.

Results of the search
We searched Cochrane Kidney and Transplant’s Specialised Register to 25 January 2016 through contact with the Information Specialist using search terms relevant to this review. Additionally, reference lists of review articles, relevant studies and clinical practice guidelines were also searched. This comprehensive search yielded 336 records. The titles and abstracts of these 336 records were assessed and 110 articles were excluded since they did not meet the prespecified review criteria. The full texts of the remaining 220 records were reviewed; six studies (34 records) met our review criteria (Figure 1). Prior to publication three additional studies were identified and these will be assessed in a future update of this review (NCT00004495; Soleimani 2011; Tayebi-Khosroshahi 2013).

Figure 1. Flow diagram showing study selection

Included studies
We included six studies that involved 2452 participants with ESKD requiring dialysis (ASFAST 2004; Heinz 2009; HOST Study 2004; Righetti 2003; Vianna 2007; Wrone 2004). All studies were reported between 2003 and 2009.

Design
All included studies were parallel RCTs (ASFAST 2004; Heinz 2009; HOST Study 2004; Righetti 2003; Vianna 2007; Wrone 2004). Five studies were double-blinded (ASFAST 2004; Heinz 2009; HOST Study 2004; Vianna 2007; Wrone 2004) and one
study followed an open-label design (Righetti 2003).

Sample sizes
Sample sizes of included studies ranged from 88 participants (Righetti 2003) to 761 participants (HOST Study 2004).

Setting
The included studies were conducted in outpatient dialysis units (ASFAST 2004; Heinz 2009; HOST Study 2004; Righetti 2003; Vianna 2007; Wrone 2004). Two were single centre studies (Righetti 2003; Vianna 2007) and four were multicentre studies (ASFAST 2004; Heinz 2009; HOST Study 2004; Wrone 2004). Studies were conducted in the USA (HOST Study 2004; Wrone 2004), Australia and New Zealand (ASFAST 2004), Germany (Heinz 2009), Brazil (Vianna 2007) and Italy (Righetti 2003). Mandatory folate fortification was present only in the USA at the time the studies were conducted. Three studies included ESKD patients on maintenance haemodialysis (Heinz 2009; Righetti 2003; Vianna 2007) only. Three studies included ESKD patients on both maintenance haemodialysis and peritoneal dialysis (ASFAST 2004; HOST Study 2004; Wrone 2004). Proportions of peritoneal dialysis patients were 28% (ASFAST 2004), 8.2% (Wrone 2004) or not reported (HOST Study 2004).

Participants
The mean age of study participants was between 48 and 65 years (Characteristics of included studies). The proportion of study participants who were male ranged from 50% to 98%, the proportion with a diagnosis of diabetes mellitus ranged from 19% to 55%, and the proportion with a history of cardiac disease ranged from 11% to 58%. Follow-up ranged from 2.0 to 3.6 years. Participants consisted entirely of people with ESKD in four studies (Heinz 2009; Righetti 2003; Vianna 2007; Wrone 2004) and both ESKD and CKD in two studies (ASFAST 2004; HOST Study 2004). Specific outcome data on ESKD patients were obtained from the study authors of ASFAST 2004.

Interventions
Interventions investigated were folic acid with or without other vitamins (vitamin B6, vitamin B12). The dose of folic acid was variable from the equivalent daily dose of 5 mg to 40 mg. Wrone 2004 compared placebo versus low dose (5 mg) versus high dose (15 mg) folate therapy with the latter two arms combined for the purposes of this review. Comparator treatment was placebo (ASFAST 2004; HOST Study 2004; Vianna 2007), low dose folic acid (Heinz 2009; Wrone 2004) or usual care (Righetti 2003).

Outcomes
For ASFAST 2004, the primary outcomes were change in rate of progression of mean maximum carotid artery intimal media thickness, composite of MI, stroke, and death from cardiovascular cause. Secondary outcomes were all fatal and nonfatal cardiovascular events including MI, stroke, unstable angina, revascularisation, and peripheral vascular disease. The primary outcome in Heinz 2009 was overall mortality. Secondary outcomes were occurrence of first fatal or nonfatal cardiovascular event (MI, unstable angina, coronary vascularisation procedure, sudden cardiac death, stroke, peripheral artery disease, PE, and thromboses). Shunt thromboses were not regarded as an end point.

The primary outcome in HOST Study 2004 was time to death from any cause. Secondary outcomes were time to MI, stroke, amputation of all or part of a lower extremity, and a composite of these three plus all-cause mortality.

For Righetti 2003, the primary outcome was a composite cardiovascular end point (typical history of angina with abnormal myocardial scintigraphy or coronarography, fatal and nonfatal MI, symptomatic extracranial carotid stenosis resulting in carotid endarterectomy, fatal and nonfatal stroke and sudden cardiac arrest). There were no secondary outcomes.

The primary outcome in Vianna 2007 was a composite of new major cardiovascular events, including death from cardiovascular causes, nonfatal MI, cardiac arrhythmias, angina, heart failure, and cerebral vascular accident. Secondary outcome was the evaluation of the intima-media thickness of the common carotid arteries. For Wrone 2004, the primary outcomes were cardiovascular events (coronary artery intervention, MI, stroke, transient ischaemic attack, carotid endarterectomy, limb amputation) and mortality. The secondary outcome was vascular access thrombosis (among those with AV fistulae).

Excluded studies
Excluded studies are described (Characteristics of excluded studies). Reasons for exclusion were: wrong study design (4 studies); less than 100 patient-years follow-up (70 studies); wrong study population (3 studies); not homocysteine-lowering interventions (19 studies); outcome data were not extractable (7 studies).

Risk of bias in included studies
A risk of bias graph and summary are presented in Figure 2 and Figure 3. Risk of selection bias related to random sequence generation and allocation concealment was low in 50% of the included studies. Risks attributed to performance, attrition, detection, and reporting biases were low in more than 65% of included studies.
Figure 2. Risk of bias graph: review authors’ judgements about each risk of bias item presented as percentages across all included studies.

Random sequence generation (selection bias)  
Allocation concealment (selection bias)  
Blinding of participants and personnel (performance bias)  
Blinding of outcome assessment (detection bias)  
Incomplete outcome data (attrition bias)  
Selective reporting (reporting bias)  
Other bias

[Legend: Low risk of bias, Unclear risk of bias, High risk of bias]
Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.
Allocation

Method of sequence generation was assessed as being at low risk of bias in three studies (ASFAST 2004; HOST Study 2004; Righetti 2003) and unclear in three studies (Heinz 2009; Vianna 2007; Wrone 2004). Allocation concealment was assessed as being at low risk of bias in three studies (ASFAST 2004; Righetti 2003; Wrone 2004) and unclear in three studies (Heinz 2009; HOST Study 2004; Vianna 2007).

Blinding

Performance bias (participants and study staff) was assessed as low in five studies (ASFAST 2004; Heinz 2009; HOST Study 2004; Vianna 2007; Wrone 2004) and high in one study (Righetti 2003). Detection bias (outcome assessors) was assessed as low in four studies (ASFAST 2004; Heinz 2009; HOST Study 2004; Vianna 2007) and unclear in two studies (Righetti 2003; Wrone 2004).

Incomplete outcome data

Attrition bias was assessed as low in all six studies.

Selective reporting

Reporting bias was assessed as low in five studies (ASFAST 2004; Heinz 2009; HOST Study 2004; Vianna 2007; Wrone 2004) and unclear in one study (Righetti 2003).

Other potential sources of bias

Righetti 2003 was assessed as being at high risk of other bias due to the imbalance between the groups in the baseline data; all other studies were assessed as being at low risk of other potential biases. Funnel plot analysis did not show publication bias (Figure 4).

Figure 4. Funnel plot of comparison: 2 Secondary outcomes, outcome: 2.1 all-cause mortality
**Effects of interventions**

**Primary outcomes**

**Cardiovascular mortality**

Four studies (ASFAST 2004; Heinz 2009; Righetti 2003; Vianna 2007) reported 173 cardiovascular mortality events among 1186 participants. Homocysteine-lowering therapy had no overall effect on cardiovascular mortality (Analysis 1.1 (4 studies, 1186 participants): RR 0.93, 95% CI 0.70 to 1.22; $I^2 = 0\%$). There was no evidence of heterogeneity.

We planned to calculate the number of persons needed to treat to avoid one cardiovascular death if adequate data were available; however, our non-significant results did not enable this calculation to be made.

**Secondary outcomes**

**All-cause mortality**

The six included studies reported 719 all-cause mortality events among 2447 participants. Homocysteine-lowering therapy had no overall effect on all-cause mortality (Analysis 2.1 (6 studies, 247 participants): RR 1.00, 95% CI 0.89 to 1.11; $I^2 = 0\%$). There was no evidence of heterogeneity.

**Fatal and nonfatal myocardial infarction**

Four studies (ASFAST 2004; Heinz 2009; Righetti 2003; Wrone 2004) reported 75 fatal and nonfatal MI events among 1510 participants. Homocysteine-lowering therapy had no overall effect on MI (Analysis 2.2 (4 studies; 1510 participants): RR 1.04, 95% CI 0.66 to 1.62; $I^2 = 0\%$). There was no evidence of heterogeneity.

**Coronary revascularisation**

Heinz 2009 and Wrone 2004 reported 44 coronary revascularisation events among 1160 participants. Homocysteine-lowering therapy had no overall effect on coronary revascularisation (Analysis 2.3 (2 studies, 1160 participants): RR 0.83, 95% CI 0.22 to 3.14; $I^2 = 74\%$). Significant heterogeneity was observed.

**Stroke**

Four studies (ASFAST 2004; Heinz 2009; Righetti 2003; Wrone 2004) reported 77 stroke events among 1510 participants. Homocysteine-lowering therapy had no overall effect on stroke (Analysis 2.4 (4 studies, 1510 participants): RR 0.89, 95% CI 0.57 to 1.40; $I^2 = 0\%$). There was no evidence of heterogeneity.

**Cerebrovascular revascularisation**

Adequate data on this outcome were not available in published reports and further information was not forthcoming from the trialists.

**Lower limb amputation**

Adequate data on this outcome were not available in published reports and further information was not forthcoming from the trialists.

**Deep vein thrombosis and pulmonary embolism**

Heinz 2009 reported 13 DVT and PE events among 650 participants. Homocysteine-lowering therapy had no overall effect on DVT and PE (Analysis 2.5: RR 1.15, 95% CI 0.39 to 3.39).

**Thrombosis of dialysis access**

HOST Study 2004 and Wrone 2004 reported 533 dialysis access thrombosis events among 1261 participants. Homocysteine-lowering therapy had no overall effect on dialysis access thrombosis (Analysis 2.6 (2 studies, 1261 participants): RR 1.00, 95% CI 0.88 to 1.14; $I^2 = 0\%$). There was no evidence of heterogeneity.

**Adverse events from folic acid-based therapy**

Adverse event rates were reported in three studies (Heinz 2009; Righetti 2003; Wrone 2004, 1248 participants) where homocysteine-lowering therapy had no effect on adverse event rates (Analysis 2.7 (3 studies, 1248 participants): RR 1.12, 95% CI 0.51 to 2.47; $I^2 = 0\%$). Reported adverse events included nausea, abdominal discomfort, hunger and weight gain, skin rashes, headaches, fatigue, and paraesthesia. We were unable to complete planned separate analyses of gastrointestinal, dermatological, neurological and malignant events due to the small number of studies reporting these outcomes. Rate of withdrawal from study treatment due to adverse events was less than 1% among the included studies.

**Subgroup analyses**

Subgroup analyses were conducted to explore possible sources of heterogeneity according to the following characteristics.

**Study intervention**

The impact of intervention on cardiovascular events did not differ significantly ($P = 0.649$) when studies of folic acid alone (ASFAST 2004; Vianna 2007) were compared with studies of folic acid plus B group vitamins (Heinz 2009; Righetti 2003).
Exposure to folic acid among control group participants
The impact of intervention on cardiovascular events did not differ significantly (P = 0.557) when studies of low dose exposure in control group participants (Heinz 2009) were compared with studies of no low dose exposure in control group participants (ASFAST 2004; Righetti 2003; Vianna 2007).

Proportion of participants with diabetes mellitus
The impact of intervention on cardiovascular events did not differ significantly (P = 0.557) when studies that included more than a third of participants with diabetes mellitus (Heinz 2009) were compared with studies that included fewer than a third of participants with diabetes mellitus (ASFAST 2004; Righetti 2003; Vianna 2007).

Proportion of participants with cardiac disease
The impact of intervention on cardiovascular events did not differ significantly (P = 1.000) when studies that included more than a third of participants with cardiac disease (Heinz 2009; Righetti 2003) were compared with studies that included fewer than a third of participants with cardiac disease (ASFAST 2004; Vianna 2007).

Study follow-up duration
The impact of intervention on cardiovascular events did not differ significantly (P = 0.649) when studies with more than 30 month follow-up (ASFAST 2004) were compared with those that had with less than 30 months follow-up (Heinz 2009; Vianna 2007; Righetti 2003).

Study event number
The impact of interventions on cardiovascular events did not differ significantly (P = 0.557) when studies with more than 150 events (Heinz 2009) were compared with those that had fewer than 150 events (ASFAST 2004; Vianna 2007; Righetti 2003). We aimed to analyse the ability of interventions to normalise homocysteine level in a subgroup of studies that enrolled patients with elevated homocysteine levels. However, there was only one study (Vianna 2007) in which a significant proportion of patients in the active arm achieved normalisation of homocysteine levels. In this study, there was no difference in cardiovascular mortality between active and control arm participants.

Sensitivity analyses
The same results were observed with fixed-effects and random-effects models.

Plausible explanations for variations in treatment effect for primary outcome were explored using subgroup analyses based on study quality and length of follow-up. The impact of intervention on cardiovascular mortality did not differ significantly (P = 0.657) when studies with and without blinding of participants and study staff (ASFAST 2004; Heinz 2009; Vianna 2007) were compared. The impact of interventions on cardiovascular mortality did not differ significantly (P = 0.557) when studies with median follow-up over three years (ASFAST 2004) were compared with median follow-up of fewer than three years.

DISCUSSION

Summary of main results
In this systematic review including 2452 participants with ESKD, randomisation to folic acid-based homocysteine-lowering therapy did not affect cardiovascular mortality, all-cause mortality, and incidence of cardiovascular events including MI, stroke, venous or access thrombosis. Null results were consistently observed across all prespecified subgroups and sensitivity analyses. Reported adverse events from these therapies were mild and rates of adverse events were variable across the studies. Overall, there was no evidence of harm defined by rates of adverse events in this population.

Overall completeness and applicability of evidence
Guidelines for ESKD patients routinely suggest supplementation with folic acid and B group vitamins (homocysteine-lowering therapies) without lack of clear evidence (K/DOQI 2005), and many people with ESKD appear to be receiving folic acid supplementation (Andreucci 2004). Our findings suggest that folic acid-based homocysteine-lowering should not be used for cardiovascular prevention in people with kidney disease, a population in whom medication burden is often high.

Quality of the evidence
The strengths of our systematic review were that an important clinical question has been investigated focusing on a study population with ESKD, using rigorous methodology and study search strategy techniques. We found consistent null results in primary, secondary, subgroup and sensitivity analyses. These findings have direct implications for millions of people with ESKD globally who are currently taking homocysteine-lowering medications. Risk of selection bias related to random sequence generation and allocation concealment was low in 50% of the included studies.
Risks attributed to performance, attrition, detection, and reporting biases were low in more than 65% of included studies.

**Potential biases in the review process**

Limitations of our systematic review include reliance on tabular rather than individual patient level data. We focused on cardiovascular mortality as our primary outcome; however, this outcome was reported in only four of the six included studies. Competing risks for cardiovascular mortality were not analysed. Although funnel plot analysis suggested a low possibility of publication bias, this cannot be excluded based on the small number of included studies. We could not conduct separate analyses for peritoneal and haemodialysis patients since adequate data were not available. Overall, participants from the included studies were younger than the average ESKD population and this may limit generalisability. Data on genetic polymorphisms that determine homocysteine-lowering effects of therapies (Kerk 2002) were not available; however, these polymorphisms have been shown to have no effects among dialysis patients (Aucella 2005). Nevertheless, although lowering of homocysteine levels were noted in all studies, normalisation of homocysteine levels was rare, pointing to the possibility of folic acid resistance in ESKD patients (Robinson 1996) and possible need for higher doses. However, in studies where high dose (> 5 mg daily) folic acid was administered, similar null effects on outcomes were noted (ASFAST 2004; HOST Study 2004; Wrone 2004).

Recent data indicate that there may be non-cardiovascular benefits of homocysteine lowering in the general population (such as reduced fracture risk) (Yang 2012) and our review did not evaluate effects on such outcomes.

**Agreements and disagreements with other studies or reviews**

Our findings were consistent with a recent meta-analysis conducted in the general population for cardiovascular mortality, all-cause mortality and MI (Huang 2012); however, our findings were discordant in terms of effects on stroke risk reduction. Huang 2012 pooled data from 19 studies that included 47,921 participants and observed approximately 12% reduction in stroke risk in the general population. Although the analyses by Huang 2012 suffer from heterogeneity of patient populations in the pooled studies, other possible reasons for differences in stroke risk reduction between general and ESKD populations may relate to differences in biological risk factors for cerebrovascular disease in dialysis versus general populations (Kanbay 2010).

VITATOPS 2010 was conducted in patients who had experienced a recent stroke or transient ischaemic attack and found no significant difference for cardiovascular events (RR 0.91, 95% CI 0.82 to 1.00, P = 0.05) or stroke (RR 0.92, 95% CI 0.81 to 1.06). There have been two recent meta-analyses that evaluated the effects of homocysteine lowering therapies on cardiovascular events in patients with kidney disease. Jardine 2012 included 11 studies comprising 4389 patients with CKD, 2452 with ESKD, and 4110 with functioning kidney transplants. In Jardine 2012, folic acid-based therapy did not reduce cardiovascular event incidence (RR 0.97, 95% CI 0.92 to 1.03, P = 0.326). Pan 2012 pooled 10 studies involving 4836 participants with CKD or ESKD. The investigators noted that the estimated relative risks were not significantly different for any cardiovascular outcomes and all-cause mortality.

Our findings were consistent with these findings. In addition, we specifically derived outcome data on ESKD patients from studies that involved both CKD and ESKD patients (ASFAST 2004; HOST Study 2004). Considering these differences, we believe that the findings of our review are likely to be a better estimate of the true effect of folic acid-based homocysteine-lowering on cardiovascular outcomes in ESKD patients. In combination with results from Jardine 2012 and Pan 2012 we feel confident regarding our conclusions on null effect from homocysteine-lowering therapies in ESKD patients and further solidify futility of such treatments as discussed in a recent editorial (Haynes 2012).

Recent data regarding associations between hyperhomocysteinaemia and cardiovascular risk (Menon 2006; Sultman 2007) have questioned the earlier findings that linked hyperhomocysteinaemia with increased cardiovascular risk (Robinson 1996) and thus further strengthen our conclusions. In fact, hyperhomocysteinaemia in ESKD may be an illustration of reverse epidemiology with some authors finding elevated homocysteine levels associated with reduced cardiovascular risk (Kalantar-Zadeh 2004) as has been described for factors such as obesity (Kalantar-Zadeh 2005) in the population with ESKD. Regardless of the nature of the association reported by observational studies, our results clearly indicate that interventions to lower homocysteine levels in people with ESKD do not offer cardiovascular risk reduction and are likely to be futile. We found one study listed at clinicaltrials.gov with unknown recruitment status on this topic (NCT00004495); however, the enrolment goal for this study is listed as 84 and it is unlikely to significantly affect results of our analyses.

**Authors’ conclusions**

**Implications for practice**

In patients with ESKD, homocysteine-lowering therapies do not reduce mortality (cardiovascular and all-cause) or cardiovascular events. Homocysteine-lowering therapies should not be used in the ESKD population for cardiovascular risk reduction.

**Implications for research**

From this review and meta-analyses, we conclude that there is no benefit in terms of cardiovascular health and mortality from ho-
mocysteine-lowering therapies in ESKD patients. The lack of benefit is consistent across various subgroups and in sensitivity analyses. Future research efforts in ESKD should focus on alternative therapies for the prevention of cardiovascular events.

ACKNOWLEDGEMENTS

We would like to thank the referees for their comments and feedback during the preparation of this review.

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HOST Study 2004 {published data only}


Vianna 2007  [published data only]

References to studies excluded from this review

Ali 2003  [published data only]

Alvares Delfino 2007  [published data only]

Anderson 2006  [published data only]

Ardalan 2003  [published data only]

Ardalan 2003a  [published data only]

Armada 2003  [published data only]

Arnadottir 2003  [published data only]

ATIC Study 2005  [published data only]


Azadibakhsh 2007  [published data only]
Azadibakhsh N, Hosseini RS, Azabak S, Nateghiyan N, Golestan B, Rad AH. Efficacy of folate and vitamin B12...


Beavers 2008 [published data only]


Bennett-Richards 2002 [published data only]

Bostom 1996 [published data only]

Bostom 2000 [published data only]


Branley 2000 [published data only]

Brensing 2002 [published data only]

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Dierkes 1999 [published data only]

Dierkes 2001 [published data only]

DIVINe Study 2010 [published data only]

Dobronzavov 2008 [published data only]

Ducloux 2002 [published data only]


Elian 2002 [published data only]

Friedman 2003 [published data only]

Galli 2003 [published data only]


Gonin 2003 [published data only]


Gonin 2003a [published data only]

Hauser 2001 [published data only]


Henning 2001 [published data only]

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Interventions for lowering plasma homocysteine levels in dialysis patients (Review)

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ISRCTN22151635 [published data only]

Jara 2001 [published data only]

Kazory 2008 [published data only]

Klemm 2004 [published data only]

Kooskhi 2011 [published data only]


Koyama 2002 [published data only]

Koyama 2010 [published data only]


Kuhlmann 2004 [published data only]

Kumar 2007b [published data only]

Kuo 2001a [published data only]

LANDMARK Study 2006 [published data only]


Johnson DW, Armstrong K, Campbell SB, Mudge DW, Hawley CM, Coombes JS, et al. Metabolic


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Madsen 2011 *(published data only)*


Manns 2001 *(published data only)*


Mazdeh 2005 *(published data only)*


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Mudge 2005 *(published data only)*


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Muller 2001 *(published data only)*


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**Nascimento 2010** *published data only*


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Rasmussen LE, Svensson M, Jorgensen KA, Schmidt EB, Christensen JH. The content of docosahexaenoic acid in serum phospholipid is inversely correlated with plasma homozygote level in patients with end-stage renal disease. *Nutrition Research* 2010;30(8):335–40. [MEDLINE: 20851307]


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**Pakfetrat 2013** *published data only*


**Pastore 2006** *published data only*


**Peng 2005** *published data only*


**Polkinghorne 2003** *published data only*

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Sepe 1999 [published data only]

Shemin 2001 [published data only]

Signorelli 2006 [published data only]

Shemin 2001 [published data only]


Sanchez Alvarez 2005 [published data only]


Scholze 2004 [published data only]


Seo 2003 [published data only]

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**Thaha 2006** *(published data only)*  

**Thaha 2008** *(published data only)*  

**Thambyrajah 2000** *(published data only)*  

**Thaha 2009** *(published data only)*  

**Thambyrajah 2000** *(published data only)*  

**Thambyrajah 1999** *(published data only)*  

**Tobe 1999** *(published data only)*  

**Tobe 2004** *(published data only)*  

**Tochihara 2008** *(published data only)*  

**Treleaven 2001** *(published data only)*  

**Tremblay 2000** *(published data only)*  

**Trimarchi 2002** *(published data only)*  

**Tungkasereerak 2006** *(published data only)*  

**Tungkasereerak 2006** *(published data only)*  

**Tungkasereerak 2006** *(published data only)*  

**Urquhart 2008** *(published data only)*  

**van Guldener 1998** *(published data only)*  
van Guldener C, Janssen M, Lambert J, ter Wee P, Donker A, Stehnoer C. Treatment of hyperhomocysteinemia with folic acid does not ameliorate endothelial function in chronic haemodialysis patients [abstract]. *Nephrology*  

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**Interventions for lowering plasma homocysteine levels in dialysis patients (Review)**  
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(Program & Abstracts):421A. [CENTRAL: CN–00689429; ]

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Tayebi-Khosroshahi 2013 {published data only}

Additional references

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Go 2004

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Haynes R, Clarke R. Homocysteine, the kidney, and vascular disease. *BMJ* 2012;344:e3925. [MEDLINE: 22695904]

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Interventions for lowering plasma homocysteine levels in dialysis patients (Review)

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Menon 2006

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Pan 2012

Robinson 1996

Suliman 2007

USRDS 2007

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van Guldener C. Why is homocysteine elevated in renal failure and what can be expected from homocysteine-lowering?. *Nephrology Dialysis Transplantation* 2006;21(5):1161–6. [MEDLINE: 16490741]

VITATOPS 2010

Wald 2002

Weiner 2004

Wrone 2001
Yang 2012

Zoccali 2010

References to other published versions of this review

Nigwekar 2009

* Indicates the major publication for the study
### Characteristics of included studies  [ordered by study ID]

#### ASFAST 2004

<table>
<thead>
<tr>
<th><strong>Methods</strong></th>
<th><strong>Characteristics</strong></th>
</tr>
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<tbody>
<tr>
<td><strong>Study design:</strong> parallel RCT</td>
<td></td>
</tr>
<tr>
<td><strong>Study duration:</strong> 30 June 1998 to 31 December 2000, median follow-up 3.6 years</td>
<td></td>
</tr>
<tr>
<td><strong>Completeness of follow-up-specific information regarding dialysis population not available; however, overall 4 patients of 315 were lost to follow-up</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Withdrawal of consent-specific information regarding dialysis population not available; however, overall 32 patients of 315 withdrew consent</strong></td>
<td></td>
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<thead>
<tr>
<th><strong>Participants</strong></th>
<th><strong>Characteristics</strong></th>
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<tbody>
<tr>
<td><strong>Setting:</strong> 5 outpatient dialysis units</td>
<td></td>
</tr>
<tr>
<td><strong>Country:</strong> Australia and New Zealand</td>
<td></td>
</tr>
<tr>
<td><strong>Relevant health status:</strong> CKD of any cause, SCr ≥ 0.40 mmol/L and awaiting commencement of dialysis or on CAPD, intermittent PD or HD</td>
<td></td>
</tr>
<tr>
<td><strong>Number:</strong> treatment group (136); control group (131)</td>
<td></td>
</tr>
<tr>
<td><strong>Mean age ± SD (years) (specific information regarding dialysis population not available): treatment group (56 ± 13); control group (56 ± 14)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Sex (male) (specific information regarding dialysis population not available): treatment group (73%); control group (62%)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Exclusion criteria:</strong> inability to obtain informed consent; inability to comply with study protocol; planned early living-related transplantation; presence of a life threatening disease such as cancer; state of high cell turnover such as inflammatory bowel disease; ongoing treatment with methotrexate; phenytoin, or trimethoprim-sulphamethoxazole; recent return to dialysis after transplantation and still on immunosuppression; cobalamin deficiency without replacement; previous bilateral carotid artery surgery or carotid artery stenosis &gt; 75% on screening</td>
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<table>
<thead>
<tr>
<th><strong>Interventions</strong></th>
<th><strong>Characteristics</strong></th>
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<tbody>
<tr>
<td><strong>Treatment group</strong></td>
<td></td>
</tr>
<tr>
<td>Folic acid</td>
<td></td>
</tr>
<tr>
<td>15 mg oral daily for median duration of 3.6 years</td>
<td></td>
</tr>
<tr>
<td><strong>Control group</strong></td>
<td></td>
</tr>
<tr>
<td>Identical appearing placebo daily for median duration of 3.6 year</td>
<td></td>
</tr>
<tr>
<td><strong>Co-interventions</strong></td>
<td>None</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Outcomes</strong></th>
<th><strong>Characteristics</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in rate of progression of mean maximum carotid artery intimal media thickness, composite of MI, stroke, and death from cardiovascular cause</td>
<td></td>
</tr>
<tr>
<td>All fatal and nonfatal cardiovascular events including MI, stroke, unstable angina, revascularisation, and peripheral vascular disease</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th><strong>Notes</strong></th>
<th><strong>Characteristics</strong></th>
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<tbody>
<tr>
<td>Funding source: National Health and Medical Research Council of Australia and National Heart Foundation of New Zealand</td>
<td></td>
</tr>
<tr>
<td>Study authors were contacted and additional information obtained</td>
<td></td>
</tr>
<tr>
<td>Other: mandatory folic acid fortification was not present in Australia and New Zealand at the time of this study</td>
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**Risk of bias**
<table>
<thead>
<tr>
<th>Bias</th>
<th>Authors’ judgement</th>
<th>Support for judgement</th>
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<tbody>
<tr>
<td>Random sequence generation (selection bias)</td>
<td>Low risk</td>
<td>RCT with random sequence generation</td>
</tr>
<tr>
<td>Allocation concealment (selection bias)</td>
<td>Low risk</td>
<td>Study medication dispensed in identical containers with neither study staff nor participant aware of treatment allocation</td>
</tr>
<tr>
<td>Blinding of participants and personnel (performance bias) All outcomes</td>
<td>Low risk</td>
<td>Double blind RCT</td>
</tr>
<tr>
<td>Blinding of outcome assessment (detection bias) All outcomes</td>
<td>Low risk</td>
<td>All clinical end points were subject to an independent adjudication process by an endpoint monitoring committee. Carotid intimal-medial thickness measurements were performed in the same study laboratory in each major city centre and a single reference laboratory performed all image analyses</td>
</tr>
<tr>
<td>Incomplete outcome data (attrition bias) All outcomes</td>
<td>Low risk</td>
<td>Data on mortality and morbidity available for 99% of participants; data analysed per intention to treat</td>
</tr>
<tr>
<td>Selective reporting (reporting bias)</td>
<td>Low risk</td>
<td>All relevant outcomes reported</td>
</tr>
<tr>
<td>Other bias</td>
<td>Low risk</td>
<td>The study appears to be free of other bias</td>
</tr>
</tbody>
</table>

**Heinz 2009**

**Methods**
- Study design: parallel RCT
- Study duration: July 2002 to July 2008, median follow-up 2.1 years
- Completeness of follow-up: of 650 patients, 107 received transplant, 75 withdrew from participation, and 18 discontinued treatment because of change in dialysis centre
- Withdrawal of consent: 75 withdrew from participation

**Participants**
- Setting: 33 outpatient dialysis units
- Country: Germany
- Relevant health status: ESKD treated for at least one month by HD
- Number: treatment group (327); control group (323)
- Mean age ± SD (years): treatment group (61 ± 13); control group (61 ± 13)
- Sex (male): treatment group (58%); control group (59%)
- Exclusion criteria: acute coronary events within 6 weeks before randomisation; active malignant tumour; pregnancy; lactation; addiction to drugs or alcohol
### Interventions

**Treatment group**
- 5 mg folic acid, 50 µg B<sub>12</sub>, 20 mg B<sub>6</sub> orally after each HD session for a median follow-up of 2.1 years
- Taken under nurse supervision

**Control group**
- 0.2 mg folic acid, 4 µg B<sub>12</sub>, 1 mg B<sub>6</sub> orally after each HD session for a median follow-up of 2.1 years
- Taken under nurse supervision

### Co-interventions
- None

### Outcomes
- Total mortality
- Occurrence of first fatal or nonfatal cardiovascular event (MI, unstable angina, coronary vascularisation procedure, sudden cardiac death, stroke, peripheral artery disease, PE, and thromboses). Shunt thromboses were not regarded as an end point

### Notes
- Funding source: School of Medicine of Otto-von-Guericke University Magdeburg; Roche diagnostics, Mannheim, Germany and Fresenius Medical Care, Bad Homburg, Germany
- Study authors contacted for additional information but no additional information was obtained
- Other: mandatory folic acid fortification was not present in Germany at the time of this study

### Risk of bias

<table>
<thead>
<tr>
<th>Bias</th>
<th>Authors’ judgement</th>
<th>Support for judgement</th>
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<tr>
<td>Random sequence generation (selection bias)</td>
<td>Unclear risk</td>
<td>RCT but sequence generation unclear</td>
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<tr>
<td>Allocation concealment (selection bias)</td>
<td>Unclear risk</td>
<td>Allocation concealment not described</td>
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<tr>
<td>Blinding of participants and personnel (performance bias)</td>
<td>Low risk</td>
<td>Double blind RCT</td>
</tr>
<tr>
<td>Blinding of outcome assessment (detection bias)</td>
<td>Low risk</td>
<td>Blinding of outcome assessment not described</td>
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<tr>
<td>Incomplete outcome data (attrition bias)</td>
<td>Low risk</td>
<td>Mortality data available on all patients</td>
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<td>Selective reporting (reporting bias)</td>
<td>Low risk</td>
<td>All relevant outcomes reported</td>
</tr>
<tr>
<td>Other bias</td>
<td>Low risk</td>
<td>The study appears to be free of other bias</td>
</tr>
</tbody>
</table>
### Methods

- **Study design**: parallel RCT
- **Study duration**: recruitment from September 2001 to October 2003, median follow-up 3.2 years
  - Completeness of follow-up: specific information regarding dialysis population not available; however, of 2056 randomised patients, 169 withdrew from regular telephone follow-up but continued to allow medical record review, and 66 withdrew consent from follow-up contacts and medical record review
  - Withdrawal of consent: specific information regarding dialysis population not available; however, of 2056 randomised patients, 66 withdrew consent from follow-up contacts and medical record review

### Participants

- **Setting**: 36 participating outpatient VA medical centres
- **Country**: USA
- **Relevant health status**: ESKD on maintenance HD or PD, or with an estimated CrCl ≤ 30 mL/min and plasma homocysteine level ≥ 15 µmol/L. For this review only patients with ESKD were included
  - **Number**: treatment group (372); control group (379)
  - **Mean age ± SD (years)** (specific information regarding dialysis population not available): treatment group (65 ± 12); control group (66 ± 12)
  - **Sex (male)** (specific information regarding dialysis population not available): treatment group (98%); control group (98%)
  - **Exclusion criteria**: age < 21 years; expected life span < 6 months; pregnancy; metastatic cancer; end-stage liver disease; treatment with methotrexate; other antifolate medication or anticonvulsants; unreliable or likely noncompliant participation in another long-term RCT or unwilling or unable to give informed consent

### Interventions

**Treatment group**
- 40 mg folic acid, 100 mg vitamin B<sub>6</sub>, 2 mg vitamin B<sub>12</sub> daily for a median follow-up of 3.2 years

**Control group**
- Identical: appearing placebo daily for a median follow-up of 3.2 years

### Co-interventions

- Participants in both groups were permitted to take additional vitamins containing no more than 1 mg of folate if prescribed by their physicians as part of their routine medical care

### Outcomes

- **Time to death from any cause**
- **Time to MI, stroke, amputation of all or part of a lower extremity, and a composite of these three plus all-cause mortality**

### Notes

- **Funding source**: Co-operative Studies program, Department of Veterans Affairs Office of Research and Development. Abbott Laboratories donated the homocysteine analytic kits. The VA Palo Alto Health Care System received payment from Pam-Lab for performing chemical analyses, including the salary of a research technician and for supplies
- **Study authors contacted for additional information but no additional information was obtained**
- **Mandatory folic acid fortification was present in the USA at the time of this study**
### Risk of bias

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<th>Support for judgement</th>
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<td>Random sequence generation (selection bias)</td>
<td>Low risk</td>
<td>Random permuted block design of varying block size</td>
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<tr>
<td>Allocation concealment (selection bias)</td>
<td>Unclear risk</td>
<td>Allocation concealment not reported</td>
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<tr>
<td>Blinding of participants and personnel (performance bias)</td>
<td>Low risk</td>
<td>Double blind RCT</td>
</tr>
<tr>
<td>Blinding of outcome assessment (detection bias)</td>
<td>Low risk</td>
<td>Blinding reported</td>
</tr>
<tr>
<td>Incomplete outcome data (attrition bias)</td>
<td>Low risk</td>
<td>For primary outcome all patients were counted. For secondary outcome analyses, 32 ESKD patients (from 751) were censored due to study withdrawal</td>
</tr>
<tr>
<td>Selective reporting (reporting bias)</td>
<td>Low risk</td>
<td>All relevant outcomes reported</td>
</tr>
<tr>
<td>Other bias</td>
<td>Low risk</td>
<td>The study appears to be free of other bias</td>
</tr>
</tbody>
</table>

### Righetti 2003

**Methods**
- Study design: parallel RCT
- Study duration: January 2001 to December 2003, median follow-up 2.4 years
- Completeness of follow-up: of 114 patients, none were lost to follow-up
- Withdrawal of consent: none withdrew from participation

**Participants**
- Setting: outpatient single centre study
- Country: Italy
- Relevant health status: ESKD treated for at least 4 months by HD
- Number: treatment group (37); control group (51)
- Mean age ± SD (years): treatment group (63.9 ± 1.6); control group (65.1 ± 1.9)
- Sex (male): treatment group (65%); control group (49%)
- Exclusion criteria: treatment with theophylline, oestrogens or anti-epileptic medications

**Interventions**
- Treatment group
  - 5 mg folic acid orally daily (every other day if serum folic acid > 16.8 ng/mL) along with an oral vitamin B complex (thiamine 250 mg, pyridoxine 250 mg and cyanocobalamin 500 mg every other day) for a median follow-up of 871 days
- Control group
  - Identical appearing placebo for a median follow-up of 871 days
Co-interventions

- None

Outcomes

- Composite cardiovascular endpoint (typical history of angina with abnormal myocardial scintigraphy or coronarography, fatal and nonfatal MI, symptomatic extracranial carotid stenosis resulting in carotid endarterectomy, fatal and nonfatal stroke and sudden cardiac arrest)

Notes

- Funding source: not provided
- Study authors contacted for additional information but no additional information was obtained
- Mandatory folic acid fortification was not present in Italy at the time of this study

Risk of bias

<table>
<thead>
<tr>
<th>Bias</th>
<th>Authors’ judgement</th>
<th>Support for judgement</th>
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<td>Low risk</td>
<td>Independent person performed randomisation using a box containing blind numbers</td>
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<td>Blinding of participants and personnel (performance bias)</td>
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<td>Incomplete outcome data (attrition bias)</td>
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<td>Selective reporting (reporting bias)</td>
<td>Unclear risk</td>
<td>Composite outcome reported</td>
</tr>
<tr>
<td>Other bias</td>
<td>High risk</td>
<td>No wash out period for group A, significant differences in baseline variables</td>
</tr>
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</table>
### Methods
- Study design: parallel RCT
- Study duration: April 2003 to March 2005, follow-up 2 years
- Completeness of follow-up: no patients were lost to follow-up. All patients were alive and examined at 6 months; between 6 and 24 months, 15 patients were transplanted and 53 died
- Withdrawal of consent: 75 withdrew from participation

### Participants
- Setting: single centre outpatient study
- Country: Brazil
- Relevant health status: ESKD patients who were stable on HD 3 times/week for at least 4 months
- Number: treatment group (93); control group (93)
- Mean age ± SD (years): treatment group (49.3 ± 13.5); control group (47.6 ± 12.3)
- Sex (male): treatment group (60%); control group (58%)
- Exclusion criteria: a potential kidney transplant from a living donor in the next three months; class IV heart failure; unstable angina; indication for coronary revascularisation; recent cerebrovascular accident; cancer; and ongoing hospitalisation

### Interventions
#### Treatment group
- Oral folic acid 10 mg 3 times/week after dialysis sessions for 2 years
- Under nurse supervision

#### Control group
- Identical appearing placebo after dialysis sessions for 2 years
- Under nurse supervision

### Co-interventions
- None

### Outcomes
- Primary end point was a composite of new major cardiovascular events, including death from cardiovascular causes, nonfatal MI, cardiac arrhythmias, angina, heart failure, and cerebral vascular accident
- Secondary end point was the evaluation of the IMT of the common carotid arteries

### Notes
- Funding source: grant from Araucaria Foundation, a Parana State Government Agency
- Study authors contacted for additional information but no additional information was obtained
- Mandatory folic acid fortification was not present in Brazil at the time of this study

### Risk of bias

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<td>Allocation concealment (selection bias)</td>
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Vianna 2007  (Continued)

<table>
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<th>Double blind RCT with participants and personnel blinded</th>
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<table>
<thead>
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<th>Blinded fashion interpretation of secondary outcomes mentioned</th>
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<table>
<thead>
<tr>
<th><strong>Incomplete outcome data (attrition bias)</strong></th>
<th><strong>Low risk</strong></th>
<th>No patient lost to follow-up</th>
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<tbody>
<tr>
<td><strong>All outcomes</strong></td>
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</table>

<table>
<thead>
<tr>
<th><strong>Selective reporting (reporting bias)</strong></th>
<th><strong>Low risk</strong></th>
<th>All relevant outcomes reported</th>
</tr>
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<tbody>
<tr>
<td></td>
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</table>

<table>
<thead>
<tr>
<th><strong>Other bias</strong></th>
<th><strong>Low risk</strong></th>
<th>The study appears to be free of other bias</th>
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<tbody>
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<td></td>
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</table>

Wrone 2004

**Methods**
- Study design: parallel RCT
- Study duration: March 1998 to May 1999
- Completeness of follow-up: patients terminated study interventions for the following reasons: death (189), received kidney transplant (58), moved away (37), and return of kidney function (2). There was no differential dropout among the treatment arms. Eight patients discontinued study medication due to side effects, which included nausea, abdominal discomfort, “hunger and weight gain”
  - Withdrawal of consent: 8 patients discontinued study medication due to side effects, which included nausea, abdominal discomfort, “hunger and weight gain.” The side effects were equally distributed among the treatment arms

**Participants**
- Setting: 10 affiliated non-profit outpatient dialysis facilities
- Country: USA
- Relevant health status: patients undergoing HD or PD
- Number: treatment group 1 (176); treatment group 2 (166); control group (168)
- Mean age ± SD (years): treatment group 1 (59.8 ± 15.4), treatment group 2 (59.5 ± 15.4); control group (61.3 ± 14.6)
  - Sex (male): treatment group 1 (51.1%); treatment group 2 (48.9%), control group (50%)
  - Exclusion criteria: patients undergoing intradialytic parenteral nutrition; anticipating a living-related kidney transplant; receiving an anti-seizure medication; residing in an institution; terminally ill

**Interventions**
- Treatment group 1
  - Renal multivitamin containing 5 mg of folic acid orally once daily for a median follow-up of 2 years
- Treatment group 2
  - Renal multivitamin containing 15 mg of folic acid orally once daily for a median follow-up of 2 years
- Control group
  - Renal multivitamin containing 1 mg folic acid orally once daily for a median...
co-interventions

- All capsules contained: 12.5 mg pyridoxine, 6 µg cobalamin, 60 mg ascorbic acid, 1.5 mg thiamine, 20 mg niacinamide, 10 mg pantothenic acid, and 0.3 mg biotin

outcomes

- Cardiovascular events (coronary artery intervention, MI, stroke, transient ischaemic attack, carotid endarterectomy, limb amputation) and mortality
- Vascular access thrombosis (among those with AV fistulae)

Notes

- Funding source: Study multivitamins were provided by R&D Laboratories, Inc., Marina del Rey, CA. This donor did not contribute to study design, data collection, analysis, interpretation of the data, or the decision to approve the manuscript
- Study authors contacted for additional information but no additional information was obtained
- Mandatory folic acid fortification was present in United States at the time of this study

Risk of bias

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<td>To maintain double-blind status, neither the person performing the randomisation nor the person preparing study medication for distribution to clinical coordinators had direct contact with participants. Patients, clinicians, and study staff with patient contact did not have access to any information that could identify treatment arm</td>
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<td>Blinding of outcome assessment (detection bias)</td>
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<td>18 patients of 588 did not receive intervention and were not analysed</td>
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<td>Selective reporting (reporting bias)</td>
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</tr>
<tr>
<td>Other bias</td>
<td>Low risk</td>
<td>The study appears to be free of other bias</td>
</tr>
</tbody>
</table>
AV - arteriovenous; CAPD - continuous ambulatory peritoneal dialysis; CKD - chronic kidney disease; CrCl - creatinine clearance; ESKD - end-stage kidney disease; HD - haemodialysis; IMT - intima-media wall thickness; MI - myocardial infarction; PD - peritoneal dialysis; PE - pulmonary embolism; RCT - randomised controlled trial; SCr - serum creatinine; SD - standard deviation

Characteristics of excluded studies  [ordered by study ID]

<table>
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<th>Reason for exclusion</th>
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<td>Alvares Delfino 2007</td>
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<td>Ardalan 2003</td>
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<td>Ardalan 2003a</td>
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<td>Arnadottir 2003</td>
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<tr>
<td>Beavers 2008</td>
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<td>Treleaven 2001</td>
<td>No pre-specified outcomes</td>
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</table>
Methods  This is an RCT. Patients are stratified according to pre-study homocysteine levels (above or below average). Patients are randomised to receive placebo or one of two doses of oral folic acid, with or without pyridoxine and cyanocobalamin

Arm I: Patients receive oral placebo daily.
Arm II: Patients receive oral pyridoxine, cyanocobalamin, and oral placebo daily
Arm III: Patients receive oral pyridoxine, cyanocobalamin, and folic acid daily
Arm IV: Patients receive oral pyridoxine and cyanocobalamin plus a higher dose of folic acid daily
Arm V: Patients receive oral placebo and oral folic acid daily
Arm VI: Patients receive oral placebo and higher dose folic acid daily
Treatment continues for 8 weeks

Participants  Ages eligible for study: 21 to 89 years
Genders eligible for study: both
Accepts healthy volunteers: no
Entry criteria

Disease characteristics: diagnosis of ESKD requiring regular HD treatment 3 times weekly; baseline predialysis total homocysteine concentration in plasma greater than 16 µmol/L; no prior or concurrent pernicious anaemia; no blood smear examination showing unexplained macrocytosis

Prior/concurrent therapy

Chemotherapy: no concurrent chemotherapy for cancer

Other: no concurrent levodopa or carbidopa; no concurrent penicillamine or trimethoprim-sulphonamide combination; no concurrent antiviral therapy No concurrent anticonvulsants

Patient characteristics

Hematopoietic: HCT at least 25%

Other: not pregnant or nursing Negative pregnancy test; fertile patients must use effective contraception; no Parkinson's disease; no convulsions or epilepsy requiring treatment; no lactose intolerance or allergy to milk products; no history of allergic sensitization following administration of folic acid, pyridoxine (vitamin B<sub>6</sub>), or cyanocobalamin (vitamin B<sub>12</sub>); no vitamin B<sub>12</sub> concentration below lower limit of normal (150 pmol/L); no untreated hypothyroidism or psoriasis

Interventions

Patients are randomised to receive placebo or one of two doses of oral folate, with or without pyridoxine and cyanocobalamin

Arm I: Patients receive oral placebo daily

Arm II: Patients receive oral pyridoxine, cyanocobalamin, and oral placebo daily

Arm III: Patients receive oral pyridoxine, cyanocobalamin, and folic acid daily

Arm IV: Patients receive oral pyridoxine and cyanocobalamin plus a higher dose of folic acid daily

Arm V: Patients receive oral placebo and oral folic acid daily

Arm VI: Patients receive oral placebo and higher dose folic acid daily

Treatment continues for 8 weeks

Outcomes

Objectives

I. Compare the efficacy of two doses of folic acid in normalizing plasma total homocysteine concentration in patients with ESKD receiving regular HD therapy resulting in hyperhomocysteinaemia

II. Determine the requirement of co-supplementation with extra pyridoxine (vitamin B<sub>6</sub>) and cyanocobalamin (vitamin B<sub>12</sub>) daily in these patients

III. Assess the safety and tolerability of this therapy in these patients

Notes

Soleimani 2011
<table>
<thead>
<tr>
<th>Method</th>
<th>Participants</th>
<th>Interventions</th>
<th>Outcomes</th>
<th>Notes</th>
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ESKD - end-stage kidney disease; HCT - haematocrit; HD - haemodialysis
### DATA AND ANALYSES

#### Comparison 1. Primary outcome

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<tr>
<th>Outcome or subgroup title</th>
<th>No. of studies</th>
<th>No. of participants</th>
<th>Statistical method</th>
<th>Effect size</th>
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</thead>
<tbody>
<tr>
<td>Cardiovascular mortality</td>
<td>4</td>
<td>1186</td>
<td>Risk Ratio (M-H, Random, 95% CI)</td>
<td>0.93 [0.70, 1.22]</td>
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#### Comparison 2. Secondary outcomes

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<tr>
<th>Outcome or subgroup title</th>
<th>No. of studies</th>
<th>No. of participants</th>
<th>Statistical method</th>
<th>Effect size</th>
</tr>
</thead>
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<tr>
<td>All-cause mortality</td>
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<td>2447</td>
<td>Risk Ratio (M-H, Random, 95% CI)</td>
<td>1.00 [0.89, 1.11]</td>
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<tr>
<td>Myocardial infarction (fatal and non-fatal)</td>
<td>4</td>
<td>1510</td>
<td>Risk Ratio (M-H, Random, 95% CI)</td>
<td>1.04 [0.66, 1.62]</td>
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<tr>
<td>Coronary revascularisation</td>
<td>2</td>
<td>1160</td>
<td>Risk Ratio (M-H, Random, 95% CI)</td>
<td>0.83 [0.42, 1.41]</td>
</tr>
<tr>
<td>Stroke</td>
<td>4</td>
<td>1510</td>
<td>Risk Ratio (M-H, Random, 95% CI)</td>
<td>0.89 [0.57, 1.40]</td>
</tr>
<tr>
<td>Deep venous thrombosis and pulmonary embolism</td>
<td>1</td>
<td></td>
<td>Risk Ratio (M-H, Random, 95% CI)</td>
<td>Totals not selected</td>
</tr>
<tr>
<td>Thrombosis of dialysis access</td>
<td>2</td>
<td>1261</td>
<td>Risk Ratio (M-H, Random, 95% CI)</td>
<td>1.00 [0.88, 1.14]</td>
</tr>
<tr>
<td>Adverse events</td>
<td>3</td>
<td>1248</td>
<td>Risk Ratio (M-H, Random, 95% CI)</td>
<td>1.12 [0.51, 2.47]</td>
</tr>
</tbody>
</table>
## Analysis 1.1. Comparison 1 Primary outcome, Outcome 1 Cardiovascular mortality.

**Review:** Interventions for lowering plasma homocysteine levels in dialysis patients

**Comparison:** 1 Primary outcome

**Outcome:** 1 Cardiovascular mortality

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Folic acid</th>
<th>Control</th>
<th>Risk Ratio</th>
<th>Weight</th>
<th>Risk Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/N</td>
<td>n/N</td>
<td>M-H, Random, 95% CI</td>
<td></td>
<td>M-H, Random, 95% CI</td>
</tr>
<tr>
<td>Righetti 2003</td>
<td>4/37</td>
<td>7/51</td>
<td>5.7% 0.79 [0.25, 2.50]</td>
<td>5.7%</td>
<td>0.79 [0.25, 2.50]</td>
</tr>
<tr>
<td>Vienna 2007</td>
<td>17/93</td>
<td>21/93</td>
<td>23.3% 0.81 [0.46, 1.43]</td>
<td>23.3%</td>
<td>0.81 [0.46, 1.43]</td>
</tr>
<tr>
<td>ASFAST 2004</td>
<td>19/133</td>
<td>20/129</td>
<td>22.6% 0.92 [0.52, 1.64]</td>
<td>22.6%</td>
<td>0.92 [0.52, 1.64]</td>
</tr>
<tr>
<td>Heinz 2009</td>
<td>43/327</td>
<td>42/323</td>
<td>48.4% 1.01 [0.68, 1.50]</td>
<td>48.4%</td>
<td>1.01 [0.68, 1.50]</td>
</tr>
</tbody>
</table>

**Total (95% CI)** 590 596 100.0% 0.93 [0.70, 1.22]

Total events: 83 (Folic acid), 90 (Control)

Heterogeneity: Tau² = 0.0; Chi² = 0.48, df = 3 (P = 0.92); I² =0.0%

Test for overall effect: Z = 0.54 (P = 0.59)

Test for subgroup differences: Not applicable
## Analysis 2.1. Comparison 2 Secondary outcomes, Outcome 1 All-cause mortality.

Review: Interventions for lowering plasma homocysteine levels in dialysis patients

Comparison: 2 Secondary outcomes

Outcome: 1 All-cause mortality

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Folic acid</th>
<th>Control</th>
<th>Risk Ratio M-H, Random, 95% CI</th>
<th>Weight</th>
<th>Risk Ratio M-H, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rigetti 2003</td>
<td>6/37</td>
<td>12/51</td>
<td>1.6 %</td>
<td>0.69 [0.28, 1.67]</td>
<td></td>
</tr>
<tr>
<td>Vianna 2007</td>
<td>23/93</td>
<td>30/93</td>
<td>5.8 %</td>
<td>0.77 [0.48, 1.22]</td>
<td></td>
</tr>
<tr>
<td>Wrone 2004</td>
<td>105/342</td>
<td>56/168</td>
<td>17.4 %</td>
<td>0.92 [0.71, 1.20]</td>
<td></td>
</tr>
<tr>
<td>ASFAST 2004</td>
<td>39/133</td>
<td>37/129</td>
<td>8.6 %</td>
<td>1.02 [0.70, 1.49]</td>
<td></td>
</tr>
<tr>
<td>HOST Study 2004</td>
<td>159/372</td>
<td>158/379</td>
<td>44.3 %</td>
<td>1.03 [0.87, 1.21]</td>
<td></td>
</tr>
<tr>
<td>Heinz 2009</td>
<td>102/327</td>
<td>92/323</td>
<td>22.2 %</td>
<td>1.10 [0.86, 1.39]</td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>1304</strong></td>
<td><strong>1143</strong></td>
<td><strong>100.0 %</strong></td>
<td><strong>1.00 [0.89, 1.11]</strong></td>
<td></td>
</tr>
</tbody>
</table>

Total events: 434 (Folic acid), 385 (Control)

Heterogeneity: $\tau^2 = 0.0$, $\chi^2 = 2.99$, df = 5 ($P = 0.70$); $I^2 = 0.0$

Test for overall effect: $Z = 0.05$ ($P = 0.96$)

Test for subgroup differences: Not applicable
Comparison 2 Secondary outcomes, Outcome 2 Myocardial infarction (fatal and non fatal).

Outcome: 2 Myocardial infarction (fatal and non fatal)

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Folic acid</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/N</td>
<td>n/N</td>
</tr>
<tr>
<td>ASFAST 2004</td>
<td>8/133</td>
<td>7/129</td>
</tr>
<tr>
<td>Heinz 2009</td>
<td>20/327</td>
<td>19/323</td>
</tr>
<tr>
<td>Righetti 2003</td>
<td>3/37</td>
<td>5/51</td>
</tr>
<tr>
<td>Wrone 2004</td>
<td>9/342</td>
<td>4/168</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>839</strong></td>
<td><strong>671</strong></td>
</tr>
</tbody>
</table>

Total events: 40 (Folic acid), 35 (Control)

Heterogeneity: $\tau^2 = 0.0, \chi^2 = 0.13, df = 3 (P = 0.99); I^2 = 0.0%$

Test for overall effect: $Z = 0.16 (P = 0.87)$

Test for subgroup differences: Not applicable
### Analysis 2.3. Comparison 2 Secondary outcomes, Outcome 3 Coronary revascularisation.

Review: Interventions for lowering plasma homocysteine levels in dialysis patients

Comparison: 2 Secondary outcomes

Outcome: 3 Coronary revascularisation

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Folic acid</th>
<th>Control</th>
<th>Risk Ratio M-H, Random, 95% CI</th>
<th>Weight</th>
<th>Risk Ratio M-H, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heinz 2009</td>
<td>8/327</td>
<td>18/323</td>
<td></td>
<td>53.7%</td>
<td>0.44 [0.19, 1.00]</td>
</tr>
<tr>
<td>Wrone 2004</td>
<td>14/342</td>
<td>4/168</td>
<td></td>
<td>46.3%</td>
<td>1.72 [0.57, 5.14]</td>
</tr>
</tbody>
</table>

**Total (95% CI)**

669 491

100.0% 0.83 [0.22, 3.14]

Total events: 22 (Folic acid), 22 (Control)

Heterogeneity: $\tau^2 = 0.69; \chi^2 = 3.83, df = 1 (P = 0.05); I^2 = 74%$

Test for overall effect: $Z = 0.28 (P = 0.78)$

Test for subgroup differences: Not applicable

### Analysis 2.4. Comparison 2 Secondary outcomes, Outcome 4 Stroke.

Review: Interventions for lowering plasma homocysteine levels in dialysis patients

Comparison: 2 Secondary outcomes

Outcome: 4 Stroke

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Folic acid</th>
<th>Control</th>
<th>Risk Ratio M-H, Random, 95% CI</th>
<th>Weight</th>
<th>Risk Ratio M-H, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASFAST 2004</td>
<td>10/133</td>
<td>11/129</td>
<td></td>
<td>30.1%</td>
<td>0.88 [0.39, 2.00]</td>
</tr>
<tr>
<td>Heinz 2009</td>
<td>11/327</td>
<td>15/323</td>
<td></td>
<td>34.9%</td>
<td>0.72 [0.34, 1.55]</td>
</tr>
<tr>
<td>Righetti 2003</td>
<td>1/37</td>
<td>2/51</td>
<td></td>
<td>3.6%</td>
<td>0.69 [0.06, 7.32]</td>
</tr>
<tr>
<td>Wrone 2004</td>
<td>19/342</td>
<td>8/168</td>
<td></td>
<td>31.3%</td>
<td>1.17 [0.52, 2.61]</td>
</tr>
</tbody>
</table>

**Total (95% CI)**

839 671

100.0% 0.89 [0.57, 1.40]

Total events: 41 (Folic acid), 36 (Control)

Heterogeneity: $\tau^2 = 0.0; \chi^2 = 0.76, df = 3 (P = 0.86); I^2 = 0.0%$

Test for overall effect: $Z = 0.50 (P = 0.61)$

Test for subgroup differences: Not applicable
### Analysis 2.5. Comparison 2 Secondary outcomes, Outcome 5 Deep venous thrombosis and pulmonary embolism.

Review: Interventions for lowering plasma homocysteine levels in dialysis patients

Comparison: 2 Secondary outcomes

Outcome: 5 Deep venous thrombosis and pulmonary embolism

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Folic acid</th>
<th>Control</th>
<th>Risk Ratio M-H, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heinz 2009</td>
<td>7/327</td>
<td>6/323</td>
<td>1.15 [0.39, 3.39]</td>
</tr>
</tbody>
</table>

- 0.1 0.2 0.5 1 2 5 10
- Favours folic acid Favours control
### Analysis 2.6. Comparison 2 Secondary outcomes, Outcome 6 Thrombosis of dialysis access.

Review: Interventions for lowering plasma homocysteine levels in dialysis patients

Comparison: 2 Secondary outcomes

Outcome: 6 Thrombosis of dialysis access

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Folic acid</th>
<th>Control</th>
<th>Risk Ratio</th>
<th>Weight</th>
<th>Risk Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOST Study 2004</td>
<td>166/372</td>
<td>163/379</td>
<td>65.3 %</td>
<td>1.04</td>
<td>[0.88, 1.22]</td>
</tr>
<tr>
<td>Wrone 2004</td>
<td>134/342</td>
<td>70/168</td>
<td>34.7 %</td>
<td>0.94</td>
<td>[0.75, 1.17]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>714</td>
<td>547</td>
<td>100.0 %</td>
<td>1.00</td>
<td>[0.88, 1.14]</td>
</tr>
</tbody>
</table>

Total events: 300 (Folic acid), 233 (Control)
Heterogeneity: Tau² = 0.0; Chi² = 0.49, df = 1 (P = 0.48); I² = 0.0%
Test for overall effect: Z = 0.04 (P = 0.97)
Test for subgroup differences: Not applicable

### Analysis 2.7. Comparison 2 Secondary outcomes, Outcome 7 Adverse events.

Review: Interventions for lowering plasma homocysteine levels in dialysis patients

Comparison: 2 Secondary outcomes

Outcome: 7 Adverse events

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Folic acid</th>
<th>Control</th>
<th>Risk Ratio</th>
<th>Weight</th>
<th>Risk Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heinz 2009</td>
<td>8/327</td>
<td>7/323</td>
<td>61.9 %</td>
<td>1.13</td>
<td>[0.41, 3.08]</td>
</tr>
<tr>
<td>Righetti 2003</td>
<td>1/37</td>
<td>3/51</td>
<td>12.6 %</td>
<td>0.46</td>
<td>[0.05, 4.24]</td>
</tr>
<tr>
<td>Wrone 2004</td>
<td>7/342</td>
<td>2/168</td>
<td>25.5 %</td>
<td>1.72</td>
<td>[0.36, 8.19]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>706</td>
<td>542</td>
<td>100.0 %</td>
<td>1.12</td>
<td>[0.51, 2.47]</td>
</tr>
</tbody>
</table>

Total events: 16 (Folic acid), 12 (Control)
Heterogeneity: Tau² = 0.0; Chi² = 0.91, df = 2 (P = 0.64); I² = 0.0%
Test for overall effect: Z = 0.29 (P = 0.77)
Test for subgroup differences: Not applicable

---

Interventions for lowering plasma homocysteine levels in dialysis patients (Review)  
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### Appendix 1. Electronic search strategies

<table>
<thead>
<tr>
<th>Database</th>
<th>Search terms</th>
</tr>
</thead>
</table>
| **CENTRAL** | 1. MeSH descriptor Homocysteine explode all trees  
2. MeSH descriptor Hyperhomocysteinemia, this term only  
3. homocysteine* in Clinical Trials  
4. hyperhomocysteine* in Clinical Trials  
5. (#1 OR #2 OR #3 OR #4)  
6. MeSH descriptor Renal Replacement Therapy explode all trees  
7. MeSH descriptor Renal Insufficiency, this term only  
8. MeSH descriptor Renal Insufficiency, Chronic explode all trees  
9. MeSH descriptor Kidney Failure, this term only  
10. MeSH descriptor Kidney Diseases, this term only  
11. “end-stage renal” or “end-stage kidney” or “endstage renal” or “endstage kidney” in Clinical Trials  
12. ESRF or ESKF or ESRD or ESKD in Clinical Trials  
13. “chronic kidney” or “chronic renal” in Clinical Trials  
14. CKF or CKD or CRF or CRD in Clinical Trials  
15. dialysis* in Clinical Trials  
16. haemodialysis or haemodialysis in Clinical Trials  
17. haemofiltration or haemofiltration in Clinical Trials  
18. haemodiafiltration or haemodiafiltration in Clinical Trials  
19. PD or CAPD or CCPD or APD in Clinical Trials  
20. (#6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19)  
21. (#5 AND #20) |
| **MEDLINE** | 1. exp Homocysteine/  
2. Hyperhomocysteinemia/  
3. hyperhomocysteine$.tw.  
4. homocysteine$.tw.  
5. or/1-4  
6. exp Renal Replacement Therapy/  
7. Renal Insufficiency/  
8. exp Renal Insufficiency, Chronic/  
9. Kidney Failure/  
10. Kidney Diseases/  
11. Diabetic Nephropathies/  
12. (kidney disease$ or kidney failure$ or renal disease$ or renal failure$).tw.  
13. (end-stage renal or end-stage kidney or endstage renal or endstage kidney).tw. |
Appendix 2. Risk of bias assessment tool

<table>
<thead>
<tr>
<th>Potential source of bias</th>
<th>Assessment criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random sequence generation</td>
<td>Low risk of bias: Random number table; computer random number generator; coin tossing;</td>
</tr>
<tr>
<td></td>
<td>shuffling cards or envelopes; throwing dice; drawing of lots; minimisation (minimisation</td>
</tr>
<tr>
<td></td>
<td>may be implemented without a random element, and this is considered to be equivalent to</td>
</tr>
<tr>
<td></td>
<td>being random)</td>
</tr>
</tbody>
</table>

| Random sequence generation Selection bias (biased allocation to interventions) due to inadequate generation of a randomised sequence                         | Low risk of bias: Random number table; computer random number generator; coin tossing;  |
|                                                              | shuffling cards or envelopes; throwing dice; drawing of lots; minimisation (minimisation |
|                                                              | may be implemented without a random element, and this is considered to be equivalent to |
|                                                              | being random)                                                                         |
High risk of bias: Sequence generated by odd or even date of birth; date (or day) of admission; sequence generated by hospital or clinic record number; allocation by judgement of the clinician; by preference of the participant; based on the results of a laboratory test or a series of tests; by availability of the intervention

Unclear: Insufficient information about the sequence generation process to permit judgement

Allocation concealment
Selection bias (biased allocation to interventions) due to inadequate concealment of allocations prior to assignment

Low risk of bias: Randomisation method described that would not allow investigator/participant to know or influence intervention group before eligible participant entered in the study (e.g. central allocation, including telephone, web-based, and pharmacy-controlled, randomisation; sequentially numbered drug containers of identical appearance; sequentially numbered, opaque, sealed envelopes)

High risk of bias: Using an open random allocation schedule (e.g. a list of random numbers); assignment envelopes were used without appropriate safeguards (e.g. if envelopes were unsealed or non-opaque or not sequentially numbered); alternation or rotation; date of birth; case record number; any other explicitly unsealed procedure

Unclear: Randomisation stated but no information on method used is available

Blinding of participants and personnel
Performance bias due to knowledge of the allocated interventions by participants and personnel during the study

Low risk of bias: No blinding or incomplete blinding, but the review authors judge that the outcome is not likely to be influenced by lack of blinding; blinding of participants and key study personnel ensured, and unlikely that the blinding could have been broken

High risk of bias: No blinding or incomplete blinding, and the outcome is likely to be influenced by lack of blinding; blinding of key study participants and personnel attempted, but likely that the blinding could have been broken, and the outcome is likely to be influenced by lack of blinding

Unclear: Insufficient information to permit judgement

Blinding of outcome assessment
Detection bias due to knowledge of the allocated interventions by outcome assessors

Low risk of bias: No blinding of outcome assessment, but the review authors judge that the outcome measurement is not likely to be influenced by lack of blinding; blinding of outcome assessment ensured, and unlikely that the blinding could have been broken

High risk of bias: No blinding of outcome assessment, and the outcome measurement is likely to be influenced by lack of blinding; blinding of outcome assessment, but likely that the blinding
<table>
<thead>
<tr>
<th><strong>Incomplete outcome data</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Attrition bias due to amount, nature or handling of incomplete outcome data</strong></td>
</tr>
</tbody>
</table>

**Low risk of bias:** No missing outcome data; reasons for missing outcome data unlikely to be related to true outcome (for survival data, censoring unlikely to be introducing bias); missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups; for dichotomous outcome data, the proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate; for continuous outcome data, plausible effect size (difference in means or standardised difference in means) among missing outcomes not enough to have a clinically relevant impact on observed effect size; missing data have been imputed using appropriate methods.

**High risk of bias:** Reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across intervention groups; for dichotomous outcome data, the proportion of missing outcomes compared with observed event risk enough to induce clinically relevant bias in intervention effect estimate; for continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes enough to induce clinically relevant bias in observed effect size; ‘as-treated’ analysis done with substantial departure of the intervention received from that assigned at randomisation; potentially inappropriate application of simple imputation.

**Unclear:** Insufficient information to permit judgement.

<table>
<thead>
<tr>
<th><strong>Selective reporting</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reporting bias due to selective outcome reporting</strong></td>
</tr>
</tbody>
</table>

**Low risk of bias:** 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; the study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified (convincing text of this nature may be uncommon).

**High risk of bias:** Not all of the study’s pre-specified primary outcomes have been reported; one or more primary outcomes is reported using measurements, analysis methods or subsets of the data (e.g. sub-scales) that were not pre-specified; one or more reported primary outcomes were not pre-specified (unless clear justification for their reporting is provided, such as an unexpected adverse effect); one or more outcomes of interest in the review are reported incompletely so that they cannot be entered in a meta-analysis; the study report fails to include results for a key outcome.

**Unclear:** Insufficient information to permit judgement.
Continued

<table>
<thead>
<tr>
<th>Other bias</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bias due to problems not covered elsewhere in the table</td>
<td>Low risk of bias: The study appears to be free of other sources of bias.</td>
</tr>
</tbody>
</table>

High risk of bias: Had a potential source of bias related to the specific study design used; stopped early due to some data-dependent process (including a formal-stopping rule); had extreme baseline imbalance; has been claimed to have been fraudulent; had some other problem

Unclear: Insufficient information to assess whether an important risk of bias exists; insufficient rationale or evidence that an identified problem will introduce bias

**HISTORY**

Protocol first published: Issue 1, 2004


<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 December 2008</td>
<td>New citation required and major changes</td>
<td>New authors</td>
</tr>
<tr>
<td>1 September 2008</td>
<td>Amended</td>
<td>Converted to new review format.</td>
</tr>
</tbody>
</table>

**CONTRIBUTIONS OF AUTHORS**

1. Draft the protocol: AC, MG, MJ, AK, SK, SDN, SN, TN, VP
2. Study selection: MJ, AK, SK, SDN, SN, VP
3. Extract data from studies: MJ, SK, SDN, SN, VP
4. Enter data into RevMan: MJ, SN
5. Carry out the analysis: MJ, SK, SDN, SN, TN, VP
6. Interpret the analysis: MJ, SK, SDN, SN, TN, VP, GS, SZ
7. Draft the final review: MJ, SK, SDN, SN, TN, VP
8. Disagreement resolution: AC, MG, MJ, AK, SK, SDN, SN, TN, VP, GS, SZ
9. Update the review: AC, MG, MJ, AK, SK, SDN, SN, TN, VP, GS, SZ
DECLARATIONS OF INTEREST

- Sagar U Nigwekar: none known
- Amy Kang: none known
- Sophia Zoungas: I have received speaker honoraria from Servier, MSD, Novo Nordisk, Sanofi Aventis, Johnson and Johnson and Astra Zeneca/BMS. I have served on external advisory boards for MSD, Amgen, AbbVie, Novo Nordisk, Novartis, Takeda, Sanofi Aventis and Astra Zeneca.
- Alan Cass: The Menzies School of Health Research has received unconditional research funding from AMGEN, Merck and Novartis for research in chronic kidney disease in Indigenous populations
- Martin P Gallagher: Martin Gallagher has received competitive research funding from the Royal Australasian College of Physicians and the Australian National Health and Medical Research Council in the last 36 months.
- Satyarth Kulshrestha: none known
- Sankar D Navaneethan: none known
- Vlado Perkovic: none known
- Giovanni FM Strippoli: Institutional support from Alfa-italian medicines agenda for Cedose trial funding (ESA dose); employment by Diaverum, renal service provider for dialysis
- Meg J Jardine: is supported by a NHMRC Career Development Fellowship and National Heart Foundation Future Leader Fellowship.

SOURCES OF SUPPORT

Internal sources
- No sources of support supplied

External sources
- Clinical Scientist in Nephrology award from the American Kidney Fund, USA.
  Sagar U Nigwekar

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

In the protocol, we mentioned N-acetyl cysteine as an intervention that lowers serum homocysteine levels to be considered for this review. Although it has this effect there are significant other effects including anti-oxidant effects from this intervention and since this review is focused on interventions that primarily reduce homocysteine, we decided to not include studies that evaluated N-acetyl cysteine in the ESKD setting in this review. Our search identified only four such studies (Ali 2003; Nascimento 2010; Scholze 2004; Tepel 2003; Thaha 2006).
INDEX TERMS

Medical Subject Headings (MeSH)
Renal Dialysis; Cardiovascular Diseases [etiology; mortality]; Cause of Death; Folic Acid [adverse effects; therapeutic use]; Homocysteine [blood]; Hyperhomocysteinemia [*drug therapy]; Kidney Failure, Chronic [*blood; therapy]; Myocardial Infarction [epidemiology]; Stroke [epidemiology]; Venous Thrombosis [epidemiology]; Vitamin B 12 [therapeutic use]; Vitamin B 6 [therapeutic use]; Vitamin B Complex [*therapeutic use]

MeSH check words
Aged; Female; Humans; Male; Middle Aged