BMJ

BMJ 2012;344:e3533 doi: 10.1136/bmj.e3533 (Published 13 June 2012)

RESEARCH

The effect of folic acid based homocysteine lowering on cardiovascular events in people with kidney disease: systematic review and meta-analysis

Meg J Jardine *senior research fellow and staff specialist (nephrology)*[1 2], Amy Kang *honours student and resident medical officer*[1 3], Sophia Zoungas *professorial fellow and associate professor*[1 4], Sankar D Navaneethan *assistant professor*[5], Toshiharu Ninomiya *assistant professor*[1 6], Sagar U Nigwekar *clinical and research fellow*[7], Martin P Gallagher *head of renal policy*[1 2], Alan Cass *senior director*[1], Giovanni Strippoli *adjunct associate professor and renal research coordinator*[8 9], Vlado Perkovic *acting executive director*[1]

[1]George Institute for Global Health, PO Box M201, Camperdown, NSW 2050, Australia; [2]Concord Repatriation General Hospital, Sydney, Australia; [3]Royal North Shore Hospital, Sydney; [4]School of Public Health, Monash University, Melbourne, Australia; [5]Department of Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH, USA; [6]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [7]Massachusetts General Hospital, Boston, MA, USA; [8]School of Public Health, University of Sydney; [9]Mario Negri Sud Consortium, Italy

Abstract

Objective To systematically review the effect of folic acid based homocysteine lowering on cardiovascular outcomes in people with kidney disease.

Design Systematic review and meta-analysis.

Data sources Medline, Embase, the Cochrane Library, and ClinicalTrials.gov to June 2011.

Study selection Randomised trials in people with non-dialysis dependent chronic kidney disease or end stage kidney disease or with a functioning kidney transplant reporting at least 100 patient years of follow-up and assessing the effect of folic acid based homocysteine lowering therapy. No language restrictions were applied.

Data extraction Two reviewers independently extracted data on study setting, design, and outcomes using a standardised form. The primary endpoint was cardiovascular events (myocardial infarction, stroke, and cardiovascular mortality, or as defined by study author). Secondary endpoints included the individual composite components, all cause mortality, access thrombosis, requirement for renal replacement therapy, and reported adverse events, including haematological and neurological events. The effect of folic acid based homocysteine lowering on outcomes was assessed with meta-analysis using random effects models.

Results 11 trials were identified that reported on 4389 people with chronic kidney disease, 2452 with end stage kidney disease, and 4110 with functioning kidney transplants (10 951 participants in total). Folic acid based homocysteine therapy did not prevent cardiovascular events (relative risk 0.97, 95% confidence interval 0.92 to 1.03, P=0.326) or any of the secondary outcomes. There was no evidence of heterogeneity in subgroup analyses, including those of kidney disease category, background fortification, rates of pre-existing disease, or baseline homocysteine level. The definitions of chronic kidney disease varied widely between the studies. Non-cardiovascular events could not be analysed as few studies reported these outcomes.

Conclusions Folic acid based homocysteine lowering does not reduce cardiovascular events in people with kidney disease. Folic acid based regimens should not be used for the prevention of cardiovascular events in people with kidney disease.

Introduction

People with kidney disease of any severity experience excess cardiovascular events and mortality compared with the general population. High plasma homocysteine levels increase as estimated glomerular filtration rate levels decline with the prevalence of hyperhomocysteinaemia (defined in relation to the upper limit of the reference range), reported to be 36-89%

Correspondence to: M J Jardine mjardine@georgeinstitute.org.au

Extra material supplied by the author (see http://www.bmj.com/content/344/bmj.e3533?tab=related#webextra)

Search strategy
Supplementary table and figures

in patients with chronic kidney disease depending on severity,[1-3] 70-75% in those with functioning kidney transplants,[4 5] and 85-100% in those with end stage kidney disease.[6-10] High homocysteine levels have been associated with an increased risk of cardiovascular events[11] in the general population, with a 25% lower homocysteine level associated with an 11% lower risk of coronary artery disease and a 19% lower risk of stroke.[12]

The direct relation between homocysteine levels and cardiovascular events observed in the general population has led to the hypothesis that reducing homocysteine levels could reduce the burden of cardiovascular disease. However, studies of homocysteine lowering in the general population have failed to show clear cardiovascular benefits.[13-16] Moreover, one study in people with a history of myocardial infarction suggested harm with use of a combination of folic acid, vitamin B_{12}, and vitamin B_6.[13]

The lack of benefit in the general population contrasts with that seen in people with homocysteinuria, where therapy prevents cardiovascular events.[17] A key distinction between the two populations is the level of homocysteine, which is noticeably higher (100-400 µmol/L) in people with homocysteinuria than in people with cardiovascular disease or diabetes (mean 13 µmol/L[13 14 18]). Homocysteine levels in people with kidney disease lie between those of the general population and those with classic homocysteinuria.[19] This has led to the hypothesis that homocysteine lowering may be useful in people with kidney disease, despite the lack of benefit in the broader population, and has driven the conduct of randomised trials in this patient group. A meta-analysis of eight large trials using individual patient level data found the lack of effect of homocysteine lowering to be consistent across categories of renal function.[20] That study utilised serum creatinine levels rather than an estimate of glomerular filtration rate to assess renal function and compared the impact of relatively mild differences in renal function (serum creatinine concentrations <80, 80-94, and ≥95 µmol/L).

We undertook a systematic review and meta-analysis to examine the effects of folic acid based homocysteine lowering compared with placebo or control treatments on cardiovascular events and other clinical outcomes in people with a range of severity of kidney disease, including kidney transplant recipients and those with non-dialysis dependent chronic kidney disease and end stage kidney disease.

Methods

A systematic review of the literature was carried out according to PRISMA[21] guidelines for the conduct of meta-analyses of intervention trials. We searched Medline via Ovid (1950 to June 2011), Embase (1966 to June 2011), and the Cochrane Library database (Cochrane central register of controlled trials), using relevant text words and medical subject headings that included all spellings of homocysteine, folic acid, vitamin B, cardiovascular disease, kidney disease, renal dialysis, and kidney transplantation (see supplementary file for search strategy). The search was limited to randomised clinical trials in any language. To identify any other relevant studies we manually scanned the reference lists of identified trials and review articles and searched the clinicaltrials.gov website for additional studies. When necessary we contacted authors or principal investigators for original data.

Study selection

Two reviewers (MJJ, AK) independently searched the literature, extracted data, and assessed the quality of trials using a standardised approach and a prespecified protocol (available from the corresponding author). We considered for inclusion all completed randomised controlled trials assessing the effects of folic acid based homocysteine lowering therapy on cardiovascular outcomes in people with kidney disease (including those receiving maintenance dialysis or with a functioning kidney transplant) and with a minimum of 100 patient years of follow-up. Studies with a sequential or crossover design were excluded. We considered studies to be eligible if they compared folic acid based homocysteine lowering therapy with placebo or usual care or if they compared a higher dose of folic acid based homocysteine lowering therapy with a lower dose. Chronic kidney disease was defined as the National Kidney Foundation kidney disease outcomes quality initiative chronic kidney disease stage 3-4 (that is, an estimated glomerular filtration rate of 15-59 mL/min/1.73 m^2), or as defined by study authors. End stage kidney disease was defined as an estimated glomerular filtration rate of less than 15 mL/min/1.73 m^2, the requirement for dialysis, or as defined by study authors. A functioning kidney transplant was defined as a kidney transplant in situ with no requirement for maintenance dialysis. The outcomes assessed were prespecified, and included cardiovascular events (defined as myocardial infarction, stroke, and cardiovascular death), all cause mortality, cardiovascular mortality, myocardial infarction, stroke, leg amputation, dialysis access thrombosis, and the start of renal replacement therapy among participants not requiring dialysis. Data on adverse events were collected, including gastrointestinal, dermatological, neurological, and malignant events.

Data extraction and quality assessment

Using a standardised form two reviewers (MJJ, AK) extracted data on participant characteristics (age, sex, study population, severity of kidney disease, presence of diabetes, or history of cardiac disease, defined as previous myocardial infarction or as defined by study authors), intervention characteristics, baseline and achieved mean homocysteine levels, mean duration of follow-up, and numbers of outcome events. Data were extracted according to intention to treat principles. One study in people with functioning renal transplants reported both intention to treat analyses and analyses censored for loss of transplant function,[22] therefore we used the intention to treat data in these analyses. The quality of the study report was determined by assessing concealment of treatment allocation; blinding of outcome assessors, care providers, and participants; completeness of study and follow-up; and intention to treat analysis.[23] Potential small study bias was assessed with the Harbord test[24] and represented graphically with Begg funnel plots of the natural log of the relative risk versus its standard error.[25] Disagreements on abstracted data were resolved by consensus and involvement of a third reviewer (VP) when necessary.

Data synthesis and analysis

We obtained summary estimates of overall and subgroup relative risk ratios using a random effects model. Analyses were done with the metan command using reported raw event counts where possible, or otherwise using reported relative risk estimates. The I^2 statistic was used to estimate the percentage of variability across studies attributable to heterogeneity beyond chance.[26] Subgroup heterogeneity was estimated by the Cochran C test, with studies weighted using the inverse variance method. Prespecified subgroup analyses included classification of kidney disease, study intervention (folic acid alone versus folic acid plus B group vitamins), baseline homocysteine level (more or

less than 20 µmol/L, the approximate 95th centile for homocysteine in men and women in the United States[27]), background fortification, proportion of study participants with diabetes at baseline (more or less than the approximate median), proportion of study participants with cardiac disease at baseline (more or less than the approximate median), whether the overall study population was recruited on the basis of kidney disease, average follow-up time (more or less than the approximate median, 30 months), study event number (more or less than the approximate median, 150 events), and composite definition (the prespecified definition of myocardial infarction, stroke, or cardiovascular death, or other definition according to the study authors).

We considered a P value of less than 0.05 as significant for all analyses. Statistical analyses were carried out with Stata 11.0 (Stata; TX, USA).

Results

The search yielded 359 articles, of which 36 were reviewed in full text (fig 1⇓). Eleven randomised trials provided information on 10 951 participants with kidney disease, totalling 4389 with stage 3-4 chronic kidney disease, 2452 with end stage kidney disease, and 4110 with functioning kidney transplants (table⇓). Trials were reported between 2004 and 2011, with additional data provided by the authors for one study.[8] Trial participants consisted entirely of people with end stage kidney disease for four trials,[6 9 28 29] both end stage kidney disease and chronic disease in two trials,[7 8] and people with a functioning kidney transplant in one trial.[22] Three papers reported on subgroups of chronic kidney disease from larger trials of vascular disease or diabetes,[30] survivors of myocardial infarction,[31] and survivors of stroke or transient ischaemic attack,[32] where chronic kidney disease was defined as an estimated glomerular filtration rate of less than 60 ml/min[30 31] or a serum creatinine concentration greater than 120 µmol/L.[32] One trial was carried out in people with diabetic nephropathy, defined as the coexistence of diabetes and albuminuria (minimum 300 mg/day) or proteinuria (minimum 500 mg/day).[33] One trial was excluded as it compared two different methods of homocysteine lowering therapy (50 mg intravenous 5-methyltetrahydrofolate three times a week compared with 5 mg oral folic acid daily) rather than comparing with placebo or comparing different dose intensities.[34]

The mean age of trial participants was between 48.5 and 72.2 years (table). The proportion of trial participants who were male ranged from 50% to 98% (median 64%), the proportion with a diagnosis of diabetes ranged from 11% to 100% (median 40%), and the proportion with a history of cardiac disease ranged from 11% to 100% (median 34%). Follow-up ranged from 24 to 60 months (median 38 months, table).

Baseline homocysteine levels were increased at baseline and were reduced by folic acid based therapy (table). The mean difference in homocysteine levels between treated and control participants was −4.97 µmol/L (95% confidence interval −9.44 to −0.49) in the five studies reporting the requisite data for the calculation.[8 9 22 30 33] Homocysteine levels were reduced into the normal range (≤12 µmol/L) in only two studies, both of which were carried out in populations with milder degrees of renal dysfunction.[22 30]

The interventions were predominantly based on daily oral folic acid supplementation in doses of between 2.5 mg and 40 mg (table). Cointerventions included supplementation with B group vitamins (seven trials, 9852 participants). Seven trials compared an intervention with placebo, one compared an intervention with usual care (no placebo), and three compared an intervention with low dose vitamins (table). One trial consisted of a three way comparison of high (15 mg), medium (5 mg), and low (1 mg) dose daily oral folic acid supplementation.[6] For the purposes of the current analysis the high and medium dose arms were combined and compared with the low dose arm. One trial that recruited people with a functioning kidney transplant reported both analyses on outcomes censored three months after the start of dialysis and outcomes of intention to treat analyses,[22] of which the intention to treat analyses were selected for our review. Baseline mean homocysteine levels ranged from 15.6 to 47 µmol/L. Five trials reported achieved mean homocysteine levels, which ranged from 11.9 to 22.6 µmol/L in the intervention group. Four trials were done on a background of fortification of grain with folic acid, whereas one was done with partial fortification of grain.

Effect of homocysteine lowering therapy on outcomes

Cardiovascular events

Ten studies reported 3045 cardiovascular events among 10 863 participants (fig 2⇓). Homocysteine lowering therapy had no overall effect on cardiovascular events (relative risk 0.97, 95% confidence interval 0.92 to 1.03, P=0.326). There was no evidence of heterogeneity across the included studies (I^2=0.0%, P=0.467 for heterogeneity).

Supplementary figure 2 shows the effects on secondary outcomes. Homocysteine lowering did not affect cardiovascular mortality (six trials, 5968 participants, relative risk 0.97, 95% confidence interval 0.82 to 1.16, supplementary figure 3), all cause mortality (nine trials, 8772 participants, 1.02 0.95 to 1.10, supplementary figure 4), myocardial infarction (eight trials, 8586 participants, 0.96, 0.84 to 1.11, supplementary figure 5), or stroke (eight trials, 8586 participants, 0.95, 0.75 to 1.21, supplementary figure 6), with no heterogeneity overall or between different categories of kidney disease.

Homocysteine lowering did not affect rates of access thrombosis (three trials, relative risk 0.96, 95% confidence interval 0.83 to 1.11, supplementary figure 7). In addition, homocysteine lowering therapy did not reduce rates for start of renal replacement therapy in people with non-dialysis dependent chronic kidney disease (two studies, 1543 participants, relative risk 1.05, 95% confidence interval 0.95 to 1.16) or a functioning kidney transplant (one study, 4110 participants, 1.12, 0.91 to 1.37, supplementary figure 8).

Tolerability of homocysteine lowering agents

Adverse event rates were reported by seven trials (supplementary figure 9). Homocysteine lowering had no effect on adverse event rates (relative risk 1.00, 95% confidence interval 0.92 to 1.08, P=0.93). The rates of reported adverse events varied noticeably between studies, ranging from 1.8% (adverse events leading to withdrawal from study treatment)[6] to 89.1% (adverse events including "many transient minor complaints, such as dizziness, nausea or headache").[33] Some trials reported only malignancies.[9 30] We were unable to complete planned separate analyses of gastrointestinal, dermatological, neurological, and malignant events owing to the small number of trials reporting these outcomes.

Subgroup analyses

The impact of folic acid based homocysteine lowering on cardiovascular events did not differ significantly in any of the subgroups studied (fig 3⇓). Patient characteristics examined

included kidney disease classification (P=0.785 for heterogeneity), baseline homocysteine levels (P=0.368 for heterogeneity), the proportion with diabetes (P=0.346 for heterogeneity), and the proportion with cardiac disease (P=0.741 for heterogeneity). Trial characteristics studied included the intervention (use of folic acid compared with folic acid plus B group vitamins: P=0.568 for heterogeneity), the type of study (primary chronic kidney disease versus subgroup of larger study: P=0.877 for heterogeneity), the average follow-up time (P=0.342 for heterogeneity), the number of events in the study (P=0.637 heterogeneity), and the use of a predefined cardiovascular composite endpoint (P=0.815 for heterogeneity). The effect of any exposure to folic acid in the controls was assessed through background folic acid fortification in the studied population (P=0.121 for heterogeneity) and through any exposure either by background fortification or by trial administered low dose folic acid (P=0.204 for heterogeneity). Continuous variables (baseline homocysteine levels, trial proportion with diabetes, trial proportion with cardiac disease, average follow-up time, and number of events in the study) were also explored using metaregression in sensitivity analyses, with results confirming that none of these factors had a significant impact on the overall meta-analysis (results not shown).

Quality

Trials were overall of high quality (supplementary table 1) with the method of sequence generation clear in eight of the 11 studies and explicit details of allocation concealment in 10 studies. Participants, study staff, and outcome assessors were blinded in 10 studies. There was low risk of bias as a result of outcome reporting and selective outcome reporting in all 11 trials. Evidence of small study publication bias was lacking (Harbord test P=0.850).

Discussion

In this large systematic review including over 10 000 participants with kidney disease, randomisation to folic acid based homocysteine lowering therapy did not affect the incidence of cardiovascular events, all cause mortality, cardiovascular mortality, myocardial infarction, stroke, requirement for dialysis treatment, or access thrombosis. There was no evidence of harm, defined by rates of adverse events in people with kidney disease, although this conclusion should perhaps be interpreted with caution given the noticeable variation in definitions and rates of adverse events reported in the trials. These outcomes were consistent across categories of kidney disease (end stage kidney disease, chronic kidney disease, and functioning kidney transplant), with no evidence of heterogeneity. Many people with kidney disease seem to be receiving folic acid supplementation[35] and some guidelines recommend supplementation of folic acid and B group vitamins or of water soluble vitamins in people with kidney disease on prudential grounds.[36 37] Our findings suggest that folic acid based homocysteine lowering therapy should not be used for the prevention of cardiovascular events in people with kidney disease, a population in whom drug burden is often high.

Comparison with systematic reviews of homocysteine lowering in all populations

These data are consistent with studies in the general population, where folic acid based homocysteine lowering has also not been found to prevent cardiovascular events in large randomised trials. Previous systematic reviews have analysed the effect of folic acid based homocysteine lowering in the general population as successive trials have been reported. In 2006, a meta-analysis of 12 randomised trials reported no effect on the risk of cardiovascular disease, coronary heart disease, stroke, or all cause mortality.[15] The possibility of benefit for stroke outcomes was raised in 2007 in a meta-analysis of eight trials (relative risk 0.82, 95% confidence interval 0.68 to 1.00, P=0.045).[38] More recently, the VITATOPS trial carried out in people with recent stroke or transient ischaemic attack found no significant effect on cardiovascular events (relative risk 0.91, 95% confidence interval 0.82 to 1.00, P=0.05) or stroke (0.92, 0.81 to 1.06).[32] The addition of the VITATOPS results to those of previous studies again found no reduction in cardiovascular events, stroke (0.94, 0.86 to 1.01), or myocardial infarction.[32]

An individual patient meta-analysis was published by the B-Vitamin Treatment Trialists' Collaboration in 2010 of 37 485 participants in eight trials randomised to folate containing B vitamins or to control for the prevention of vascular disease.[20] The meta-analysis showed that folic acid supplementation had no impact on major vascular events, mortality, or cancer incidence despite an average 25% reduction in homocysteine levels. There was no evidence of heterogeneity in subgroup analyses comparing the impact of the intervention according to serum creatinine concentrations (<80, 80-94, and ≥95 μmol/l). The trialists' analysis included 4361 participants contained in the current analysis, representing 12% of the individual patient data meta-analysis and 40% of the current report. The additional 6590 participants included in the current report are from studies published after the previous report (4603 participants) or from studies that did not meet the 1000 participant criteria of the former report (1987 participants). These studies have effectively excluded any beneficial cardiovascular effect of homocysteine lowering therapy in the general population, and the current review adds to this evidence by similarly excluding meaningful benefit in kidney disease.

Comparison with previous systematic reviews of homocysteine-lowering in people with kidney disease

A previous systematic review of folic acid based homocysteine lowering in people with advanced or end stage kidney disease reported a reduction in all fatal and non-fatal cardiovascular events (seven trials, 3886 participants, relative risk 0.85; 95% confidence interval 0.76 to 0.96; P=0.009) and no significant reduction in a composite of myocardial infarctions, stroke, and cardiovascular death (five trials, 3619 participants, 0.87, 0.75 to 1.00, P=0.06), with the point estimate favouring the intervention but with some imprecision.[39] Differences between the analyses include the near threefold higher number of participants in the current review (10 951 in 11 trials compared with 3886 in seven trials in the previous analysis) through the inclusion of all kidney disease categories, permitting a broader study population. Our study included all reports of outcomes for patients with kidney disease randomised to homocysteine lowering to comprehensively summate the available evidence, permitting the inclusion of the post hoc analysis of HOPE-2.[30] Additionally the two studies had methodological differences. The previous meta-analysis summated the separate reports for various cardiovascular outcomes (for example, myocardial infarction, stroke, sudden cardiac death) to derive a figure for a composite of non-fatal myocardial infarction, non-fatal stroke, or death from cardiovascular cause resulting in the potential for some participants to contribute to the outcome more than once. In our analysis we elected to use the trial definition where this study's composite was not reported. The different approach to the treatment of the composite accounts for most of the

difference between ours and the previous report, with the risk ratio falling from 0.972 (95% confidence interval 0.902 to 1.047) to 0.880 (0.762 to 1.015) in the four studies where both methods can be tested. We believe the findings of the current review are likely to be a better estimate of the true effect of folic acid based homocysteine lowering on cardiovascular outcomes. Further information and precision could be gained from analysis of all completed trials through the conduct of further analyses using data at individual patient level.

Impact of folic acid on homocysteine lowering in people with and without kidney disease

Doses of 0.8 mg of folic acid or more are required to achieve maximal homocysteine lowering of a standardised 23% reduction in trials of participants where folic acid dose was compared with homocysteine reduction.[40] The addition of vitamin B_{12} increased the degree of homocysteine lowering to 30%. In 25 trials of homocysteine lowering utilising 0.8 mg folic acid or higher, homocysteine was lowered to 12 µmol/L or less in treated participants in all but two studies, and one of these was carried out in participants with kidney disease.[41] Conversely among the trials of the current analysis, homocysteine levels were largely not normalised despite doses of folic acid substantially greater than 0.8 mg being administered, a finding that has been shown previously.[41 42] It has been postulated that the relative resistance of patients with end stage kidney disease to folic acid based therapy may be due to impaired folic acid metabolism and impaired folate absorption as well as impaired renal clearance.[43]

Observed relation of homocysteine levels with cardiovascular events in people with kidney disease

Adding further strength to these conclusions, earlier reports of clear relations between homocysteine levels and cardiovascular events in people with kidney disease[43] have been challenged by more recent studies observing no association[44 45] or even an inverse association.[10] A meta-analysis of observational studies in end stage kidney disease found no association between homocysteine levels and clinical events in retrospective studies, whereas prospective studies showed no association with all cause mortality but a positive association with cardiovascular events, which increased by 9% with every 5 µmol/L increase in homocysteine levels. These observations of the risks associated with various homocysteine levels led in part to the generation of the "reverse epidemiology hypothesis," which asserts altered risk factor patterns may be confounded by the poor nutrition and inflammatory state common in patients requiring dialysis such that the association of homocysteine and outcomes is driven by nutritional state.[46 47] Another explanation supported by some[45 48 49] but not all[5 50 51] analyses, is that homocysteine is simply a marker of kidney function. Regardless of the nature of the association reported by observational studies, it is now clear that interventions to lower homocysteine levels in people with kidney disease do not lead to a reduction in cardiovascular events.

Impact of background fortification on homocysteine lowering

The relative risk of cardiovascular events was 0.91 (95% confidence interval 0.81 to 1.02) in trials where controls had no exposure to folic acid based regimens, compared with 0.99 (0.93 to 1.06) in trials where controls did have some level of exposure, either through fortification or through comparator treatment. The difference was not significant (P=0.24 for heterogeneity). The individual patient data meta-analysis of eight large trials found that folic acid based therapy had no impact on major vascular events, whether administered against a background of fortification (relative risk 0.99 99% confidence interval 0.90 to 1.09) or not (1.02, 0.96 to 1.09).[20] Short of further randomised trials, individual patient level data analysis of participants in all homocysteine lowering trials according to kidney disease status may increase analysis power providing greater clarity.

Strengths and limitations of the review

The strengths of this systematic review are its rigorous methodology, the importance of the clinical question, and the clear result. The findings have direct implications for the management of hundreds of millions of people with kidney disease around the world, including millions receiving dialysis for end stage kidney disease, many of whom are currently taking folic acid supplements. Limitations include the reliance on tabular data rather than individual patient level data. Some studies used different definitions for the primary outcome of cardiovascular events, although this did not seem to meaningfully impact the findings. We could only include results for people with kidney disease included in larger trials where these are reported, raising the possibility of reporting bias. However this would be expected to exaggerate any benefit and none was found. Furthermore, the definition of chronic kidney disease varied among the studies (see table).

Conclusion

In summary, folic acid based homocysteine lowering does not prevent cardiovascular events in people with kidney disease and consideration should be given to discontinuing its use for cardiovascular prevention in this population.

We thank SZ and K Polkinghorne for additional data from the ASFAST study; Gail Higgins, trials search coordinator of the Cochrane Renal Group, for assistance with the search strategy and implementation; MJJ and MPG were supported by grants from the Royal Australasian College of Physicians Jacquot Research Establishment; TN was supported by a Banyu Life Science Foundation International fellowship programme (Japan) and by a Foundation of High Blood Pressure Research ISH visiting postdoctoral award (Australia). SZ was supported by a National Heart Foundation of Australia Career Development Award. VP was supported by a NSW Cardiovascular Research Network Heart Foundation of Australia fellowship. The authors were independently responsible for the study design, analysis, interpretation, preparation of the manuscript, and the decision to submit for publication.

Contributors: MJJ contributed to the study design, screened articles, extracted data, performed statistical analyses and drafted and revised the paper. AK contributed to the study design, sourced articles, screened articles, extracted data and revised the paper. SZ contributed to the study design, obtained data from one of the trials, and revised the paper. SDN contributed to the study design and revised the paper, TN contributed to the study design, performed statistical analyses and revised the paper, SUN contributed to the study design, extracted data and revised the paper, MPG contributed to the study design and revised the paper, AC contributed to the study design and revised the paper, GS contributed to the study design and revised the paper, VP initated the study, contributed to the study design and revised the paper. All authors had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding: This study received no external funding.

What is already known on this topic

Elevated homocysteine levels are associated with an increased risk of cardiovascular events

Homocysteine lowering in the general population has failed to show clear cardiovascular benefits unlike the situation with people with homocysteinuria who have noticeably increased homocysteine levels

Homocysteine levels increase as estimated glomerular filtration rate levels decline

What this study adds

In over 10 000 people with a range of severity of kidney disease no benefit was seen from folic acid based homocysteine lowering therapy for cardiovascular events, all cause mortality, cardiovascular mortality, myocardial infarction, stroke, requirement for dialysis treatment, or access thrombosis

The results were consistent across categories of kidney disease (end stage kidney disease, chronic kidney disease, and functioning kidney transplant), with no evidence of heterogeneity

Our findings suggest that folic acid based homocysteine lowering should not be used for cardiovascular prevention in people with kidney disease

Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that : no support from any organisation for the submitted work; SZ is a member of the advisory boards for MSD, Novo Nordisk, and Boehringer Ingelheim, and has received consultancies from Novo Nordisk and Johnson and Johnson, speakers fees from MSD, Novo Nordisk, Astra Zeneca/BMS, Novartis, Sanofi Aventis, and Servier International, and payment for the development of educational presentations from Medi Mark; MG has received payment for lectures from Roche Pharmaceuticals; SDN has received a KL2 grant from the National Institutes of Health; AC has received payment for lectures from Roche, Servier, AMGEN, and MSD; MJ has received an unrestricted grant from CSL; Concord Hospital has received an educational grant from Shire; VP is a member of the Abbot advisory board, has grants or grants pending from Roche, Johnson and Johnson, Baxter, and Servier and has received payment for lectures from Roche, Servier, and Astra Zeneca; MJ, AK, SZ, TN, MG, AC, and VP are affiliated with the George Institute, which receives funding from various pharmaceutical companies to support parts of its research activities, which companies might have an interest in the submitted work in the previous three years; and no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: Not required.

Data sharing: No additional data available.

1 Lin Y-H, Pao K-Y, Wu V-C, Lin Y-L, Chien Y-F, Hung C-S, et al. The influence of estimated creatinine clearance on plasma homocysteine in hypertensive patients with normal serum creatinine. *Clin Biochem* 2007;40:230-4.
2 Nerbass FB, Draibe SA, Feiten SF, Chiarello PG, Vannucchi H, Cuppari L. Homocysteine and its determinants in nondialyzed chronic kidney disease patients. *J Am Diet Assoc* 2006;106:267-70.
3 Menon V, Wang X, Greene T, Beck GJ, Kusek JW, Selhub J, et al. Homocysteine in chronic kidney disease: effect of low protein diet and repletion with B vitamins. *Kidney Int* 2005;67:1539-46.
4 Winkelmayer WC, Kramar R, Curhan GC, Chandraker A, Endler G, Fodinger M, et al. Fasting plasma total homocysteine levels and mortality and allograft loss in kidney transplant recipients: a prospective study. *J Am Soc Nephrol* 2005;16:255-60.
5 Ducloux D, Motte G, Challier B, Gibey R, Chalopin JM. Serum total homocysteine and cardiovascular disease occurrence in chronic, stable renal transplant recipients: a prospective study. *J Am Soc Nephrol.* 2000;11:134-7.
6 Wrone EM, Hornberger JM, Zehnder JL, McCann LM, Coplon NS, Fortmann SP. Randomized trial of folic acid for prevention of cardiovascular events in end-stage renal disease. *J Am Soc Nephrol* 2004;15:420-6.
7 Jamison RL, Hartigan P, Kaufman JS, Goldfarb DS, Warren SR, Guarino PD, et al. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: a randomized controlled trial. *JAMA* 2007;298:1163-70.
8 Zoungas S, McGrath BP, Branley P, Kerr PG, Muske C, Wolfe R, et al. Cardiovascular Morbidity and Mortality in the Atherosclerosis and Folic Acid Supplementation Trial (ASFAST) in chronic renal failure: a multicenter, randomized, controlled trial. *J Am Coll Cardiol* 2006;47:1108-16.
9 Righetti M, Serbelloni P, Milani S, Ferrario G. Homocysteine-lowering vitamin B treatment decreases cardiovascular events in hemodialysis patients. [see comment]. *Blood Purif* 2006;24:379-86.
10 Kalantar-Zadeh K, Block G, Humphreys MH, McAllister CJ, Kopple JD. A low, rather than a high, total plasma homocysteine is an indicator of poor outcome in hemodialysis patients. *J Am Soc Nephrol* 2004;15:442-53.
11 Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *BMJ* 2002;325:1202.
12 Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA* 2002;288:2015-22.
13 Bonaa KH, Njolstad I, Ueland PM, Schirmer H, Tverdal A, Steigen T, et al. Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N Engl J Med* 2006;354:1578-88.
14 Toole JF, Malinow MR, Chambless LE, Spence JD, Pettigrew LC, Howard VJ, et al. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. *JAMA* 2004;291:565-75.
15 Bazzano LA, Reynolds K, Holder KN, He J. Effect of folic acid supplementation on risk of cardiovascular diseases: a meta-analysis of randomized controlled trials. *JAMA* 2006;296:2720-6.
16 Mei W, Rong Y, Jinming L, Yongjun L, Hui Z. Effect of homocysteine interventions on the risk of cardiocerebrovascular events: a meta-analysis of randomised controlled trials. *Int J Clin Pract* 2010;64:208-15.
17 Yap S. Classical homocystinuria: vascular risk and its prevention. *J Inherit Metab Dis* 2003;26:259-65.
18 Lonn E, Yusuf S, Arnold MJ, Sheridan P, Pogue J, Micks M, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med* 2006;354:1567-77.
19 Heinz J, Kropf S, Luley C, Dierkes J. Homocysteine as a risk factor for cardiovascular disease in patients treated by dialysis: a meta-analysis. *Am J Kidney Dis* 2009;54:478-89.
20 Clarke R, Halsey J, Lewington S, Lonn E, Armitage J, Manson JE, et al. Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality: meta-analysis of 8 randomized trials involving 37 485 individuals. *Arch Intern Med* 2010;170:1622-31.
21 Moher D, Liberati A, Tetzlaff J, Altman DG, The PG. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: the PRISMA statement. *PLoS Med* 2009;6:e1000097.
22 Bostom AG, Carpenter MA, Kusek JW, Levey AS, Hunsicker L, Pfeffer MA, et al. Homocysteine-lowering and cardiovascular disease outcomes in kidney transplant recipients: primary results from the folic acid for vascular outcome reduction in transplantation trial. *Circulation* 2011;123:1763-70.
23 JPT Higgins, Green S (eds). Cochrane handbook for systematic reviews of interventions version 5.1.0 [updated March 2011]. Cochrane Collaboration, 2011.
24 Harbord RM, Egger M, Sterne JAC. A modified test for small-study effects in meta-analyses of controlled trials with binary endpoints. *Stat Med* 2006;25:3443-57.
25 Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;315:629-34.
26 Woodward M. Epidemiology: study design and data analysis. 2nd ed. Chapman and Hall/CRC Press, 2005.
27 Ganji V, Kafai MR. Population reference values for plasma total homocysteine concentrations in US adults after the fortification of cereals with folic acid. *Am J Clin Nutr* 2006;84:989-94.
28 Heinz JM, Kropf SP, Domrose UMD, Westphal SMD, Borucki KMD, Luley CMD, et al. B vitamins and the risk of total mortality and cardiovascular disease in end-stage renal disease: results of a randomized controlled trial. [Article]. *Circulation* 2010;121:1432-8.
29 Vianna ACA, Mocelin AJ, Matsuo T, Morais-Filho D, Largura A, Delfino VA, et al. Uremic hyperhomocysteinemia: a randomized trial of folate treatment for the prevention of cardiovascular events. *Hemodial Int* 2007;11:210-6.
30 Mann JFE, Sheridan P, McQueen MJ, Held C, Arnold JMO, Fodor G, et al. Homocysteine lowering with folic acid and B vitamins in people with chronic kidney disease—results of the renal Hope-2 study. *Nephrol Dial Transplant* 2008;23:645-53.
31 Armitage JM, Bowman L, Clarke RJ, Wallendszus K, Bulbulia R, Rahimi K, et al. Effects of homocysteine-lowering with folic acid plus vitamin B12 vs placebo on mortality and major morbidity in myocardial infarction survivors: a randomized trial. *JAMA* 2010;303:2486-94.
32 The VITATOPS Trial Study Group. B vitamins in patients with recent transient ischaemic attack or stroke in the VITAmins TO Prevent Stroke (VITATOPS) trial: a randomised, double-blind, parallel, placebo-controlled trial. *Lancet Neurol* 2010;9:855-65.
33 House A, Eliasziw M, Cattran D, Churchill D, Oliver M, Fine A, et al. Effect of B-vitamin therapy on progression of diabetic nephropathy: a randomized controlled trial. *JAMA* 2010;303:1603-9.
34 Cianciolo G, La Manna G, Coli L, Donati G, D'Addio F, Persici E, et al. 5-methyltetrahydrofolate administration is associated with prolonged survival and reduced inflammation in ESRD patients. *Am J Nephrol* 2008;28:941-8.
35 Andreucci VE, Fissell RB, Bragg-Gresham JL, Ethier J, Greenwood R, Pauly M, et al. Dialysis Outcomes and Practice Patterns Study (DOPPS) data on medications in hemodialysis patients. *Am J Kidney Dis* 2004;44(5 suppl 2):61-7.
36 KDOQI Clinical Practice Guidelines for Cardiovascular Disease in Dialysis Patients: section III. State of the science: novel and controversial topics in cardiovascular diseases. NFK KDOQI Guidelines, 2005. www.kidney.org/professionals/kdoqi/guidelines_cvd/homocystein.htm.
37 Holt S, Goldsmith D. Cardiovascular disease in CKD. Guidelines: UK Renal Association, 2010. www.renal.org/Clinical/GuidelinesSection/CardiovascularDiseaseInCKD.aspx#Summary2.

38 Wang X, Qin X, Demirtas H, Li J, Mao G, Huo Y, et al. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. *Lancet* 2007;369:1876-82.
39 Qin X, Huo Y, Langman CB, Hou F, Chen Y, Matossian D, et al. Folic acid therapy and cardiovascular disease in ESRD or advanced chronic kidney disease: a meta-analysis. *Clin J Am Soc Nephrol* 2011;6:482-8.
40 Homocysteine Lowering Trialists Collaboration. Dose-dependent effects of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials. *Am J Clin Nutr* 2005;82:806-12.
41 K/DOQI Workgroup. K/DOQI clinical practice guidelines for cardiovascular disease in dialysis patients. *Am J Kidney Dis* 2005;45(4 suppl 3):S1-153.
42 Levey AS, Eknoyan G. Cardiovascular disease in chronic renal disease. [see comment]. *Nephrol Dial Transplant* 1999;14:828-33.
43 Robinson K, Gupta A, Dennis V, Arheart K, Chaudhary D, Green R, et al. Hyperhomocysteinemia confers an independent increased risk of atherosclerosis in end-stage renal disease and is closely linked to plasma folate and pyridoxine concentrations. *Circulation* 1996;94:2743-8.
44 Nair AP, Nemirovsky D, Kim M, Geer EB, Farkouh ME, Winston J, et al. Elevated homocysteine levels in patients with end-stage renal disease. *Mt Sinai J Med* 2005;72:365-73.
45 Menon V, Sarnak MJ, Greene T, Wang X, Pereira AA, Beck GJ, et al. Relationship between homocysteine and mortality in chronic kidney disease. *Circulation* 2006;113:1572-7.
46 Kopple JD. The phenomenon of altered risk factor patterns or reverse epidemiology in persons with advanced chronic kidney failure. *Am J Clin Nutr* 2005;81:1257-66.
47 Suliman M, Stenvinkel P, Qureshi AR, Kalantar-Zadeh K, Barany P, Heimburger O, et al. The reverse epidemiology of plasma total homocysteine as a mortality risk factor is related to the impact of wasting and inflammation. *Nephrol Dial Transplant* 2007;22:209-17.
48 Potter K, Hankey GJ, Green DJ, Eikelboom JW, Arnolda LF. Homocysteine or renal impairment. which is the real cardiovascular risk factor? *Arterioscler Thromb Vasc Biol* 2008;28:1158-64.
49 Silva de Almeida CC, Guerra DC, Vannucchi MTI, Geleilete TJM, Vannucchi H, Chiarello PG. What is the meaning of homocysteine in patients on dialysis? *J Ren Nutr* 2011;21:394-400.
50 Massy ZA, Chadefaux-Vekemans B, Chevalier A, Bader CA, Drueke TB, Legendre C, et al. Hyperhomocysteinaemia: a significant risk factor for cardiovascular disease in renal transplant recipients. *Nephrol Dial Transplant* 1994;9:1103-8.
51 Connolly GM, Cunningham R, McNamee PT, Young IS, Maxwell AP. Elevated homocysteine is a predictor of all-cause mortality in a prospective cohort of renal transplant recipients. *Nephron Clin Pract* 2010;114:c5-11.

Accepted: 8 May 2012

Cite this as: *BMJ* 2012;344:e3533

Table

Table 1| Characteristics of studies reporting effect of folic acid based homocysteine lowering therapy in people with kidney disease

Study	Study population	Category of kidney disease No (%)	Mean age (years)	% male	No (%) with diabetes	No (%) with cardiac disease	Components of cardiovascular composite	Mean homocysteine* (µmol)		Study intervention‡	Comparison	Grain fortification	M fo (m
								Baseline	Achieved†				
Wrone 2004[6]	ESKD	ESKD 510 (100)	60.2	50	232 (45)	174 (34)§	Myocardial infarction, stroke, death, coronary revascularisation, transient ischaemic attack, carotid endarterectomy, amputation	32.9	24.9	15 mg or 5 mg folic acid daily¶	1 mg folic acid	Yes	
Zoungas 2006[8] (ASFAST)	ESKD and CKD	ESKD 267 (85); CKD 48 (15)**	56.2	68	73 (23)	35 (11)††	Myocardial infarction, stroke, cardiovascular death	27	21.5‡‡	15 mg folic acid	Placebo	No	
Righetti 2006[9]	ESKD (HD)	ESKD 88 (100)	64.6	56	17 (19)	51 (58)§	Myocardial infarction, stroke, sudden cardiac death, angina, carotid endarterectomy in patients with symptoms§§	34.6	22.6	Folic acid 5 mg daily or 2nd daily according to folic acid levels+vitamin B complex 2nd daily (B_1 250 mg, B_6 250 mg, B_{12} 500 mg) if plasma B_{12} depleted	Usual care	No	
Jamison 2007[7] (HOST)	ESKD and CKD	ESKD 751 (37); CKD 1305 (63)¶¶	65.8	98	1129 (55)	511 (25)††	Myocardial infarction, stroke, all cause mortality, amputation	24.1	17.8	40 mg folic acid, 100 mg vitamin B_6, 2mg vitamin B_{12} daily	Placebo	Yes	
Vianna 2007[29]	ESKD	ESKD 186 (100)	48.5	59	42 (23)	44 (24)§	Myocardial infarction, stroke, cardiovascular death, arrhythmias, angina, heart failure	25.0‡‡	NR	10 mg folic acid 3 times per week after haemodialysis	Placebo	No	
Mann 2008[30] (HOPE 2)***	Vascular disease or DM (CKD 11%)	CKD 619 (100)†††	72.2	67	269 (43)	537 (87)§	Myocardial infarction, stroke, cardiovascular death	15.8	11.9	2.5 mg folic acid, 50 mg vitamin B_6, 1 mg vitamin B_{12}	Placebo	Partial‡‡‡	
House 2010[33] (DIVINe)	Diabetic nephropathy§§§	CKD 238 (100)	60.4	75	238 (100)	74 (31.1)§	Myocardial infarction, stroke, all cause mortality, revascularisation	15.5	13.3	2.5 mg folic acid, 25 mg vitamin B_6, 1mg vitamin B_{12}	Placebo	Yes	
Armitage 2010[31] (SEARCH)***	History of myocardial infarction	CKD 1686 (100)†††	64.2¶¶¶	83¶¶¶	1267 (11)¶¶¶	1686 (100)†††¶¶¶	Myocardial infarction, stroke, coronary heart disease death, revascularisation	NR	NR	2 mg folic acid, 1 mg vitamin B_{12}	Placebo	No	80
Heinz 2010[28]	ESKD	ESKD 650 (100)	61.0	58	262 (40)	312 (48)	Myocardial infarction, stroke, sudden cardiac death, unstable angina, revascularisation, peripheral arterial disease, pulmonary embolism,	29	18.8	5 mg folic acid, 50 µg B_{12}, 20 mg B_6 after haemodialysis	0.2 mg folic acid, 4 µg B_{12}, 1 mg B_6 after haemodialysis	No	

(continued)

Study	Study population	Category of kidney disease No (%)	Mean age (years)	% male	No (%) with diabetes	No (%) with cardiac disease	Components of cardiovascular composite	Mean homocysteine* (µmol)		Study intervention‡	Comparison	Grain fortification	M fo (m
								Baseline	Achieved†				
							thromboses excluding shunt thromboses						
Hankey 2010[32] (VITATOPS)***	Stroke or TIA in preceding 7 months	CKD 493 (100)††††	62.6¶¶¶	64¶¶¶	1899 (24)¶¶¶	598 (7)‡‡¶¶¶	Myocardial infarction, stroke, cardiovascular death	NR	NR	2 mg folic acid, 25 mg vitamin B_6, 0.5 mg vitamin B_{12}	Placebo	Partial‡‡‡	4
Bostom 2011[22] (FAVORIT)	Kidney transplant recipients	KTR 4110 (100)	52	63	1663 (40.5)	820 (20.0)§	Myocardial infarction, stroke, cardiovascular disease death, resuscitated sudden death, revascularisation, above ankle amputation, repair of abdominal aortic aneurysm	16.4	11.8‡‡‡‡	5 mg folic acid, 50 mg B_6, 1 mg B_{12}	1.4 mg B_6, 2.0 µg B_{12}	Yes	4

CKD=predialysis chronic kidney disease; ESKD=dialysis dependent end stage kidney disease; KTR=functioning kidney transplant recipient; NR:=not reported.

*Homocysteine levels not included if not available for subgroup with chronic kidney disease.

†In intervention group.

‡Daily unless stated otherwise.

§Cardiac disease as defined by study author.

¶Study design had two intervention arms and one "standard care" arm. For the current analysis, two intervention arms are combined.

**Non-dialysis dependent chronic kidney disease, defined as serum creatinine concentration ≥0.4 mmol/L.

††Cardiac disease defined as previous myocardial infarction.

§§Cardiovascular composite not reported separately for randomised and observed participants.

¶¶Chronic kidney disease defined as Cockcroft-Gault estimated glomerular filtration rate ≤30 ml/min.

‡‡Median reported.

***Chronic kidney disease subgroup of larger trial.

†††MDRD estimated glomerular filtration rate <60 ml/min. Participants with severe renal disease (serum creatinine level >2× upper limit of normal) were excluded by design.

‡‡‡Grain fortification in some but not all trial countries.

§§§Participants with chronic kidney disease stage 4 and 5 were excluded by design.

¶¶¶Data for chronic kidney disease subgroup not available, data for total study reported.

****Mean reported.

††††Chronic kidney disease defined as serum creatinine concentration >120 µmol/L.

‡‡‡‡Based on a sample of 72 participants in the intervention group.

Figures

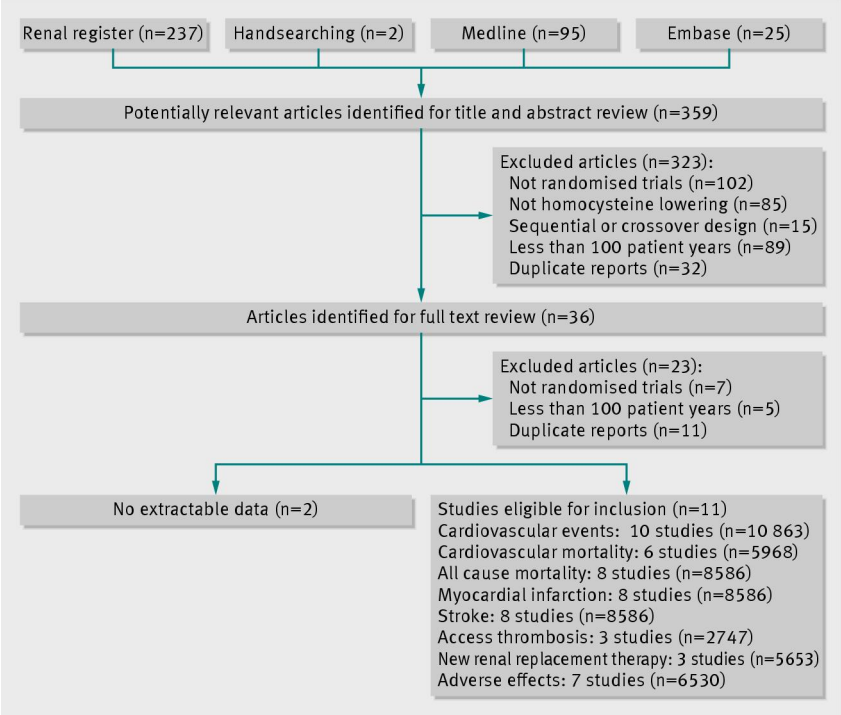

Fig 1 Identification process for eligible studies

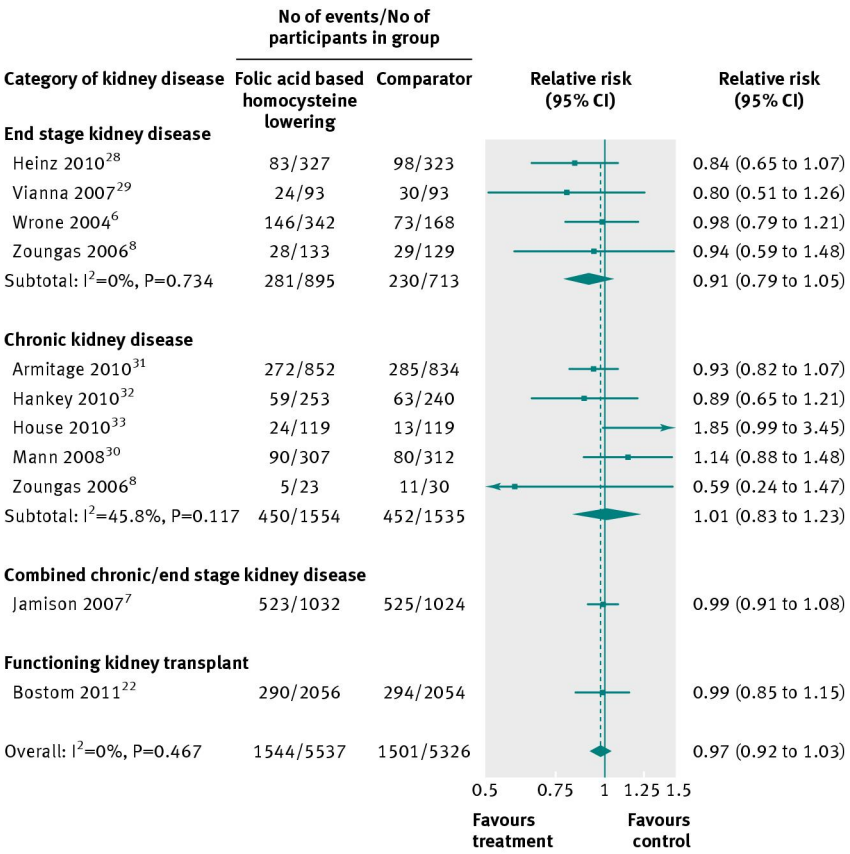

Fig 2 Effect of folic acid based homocysteine lowering therapy on composite cardiovascular events

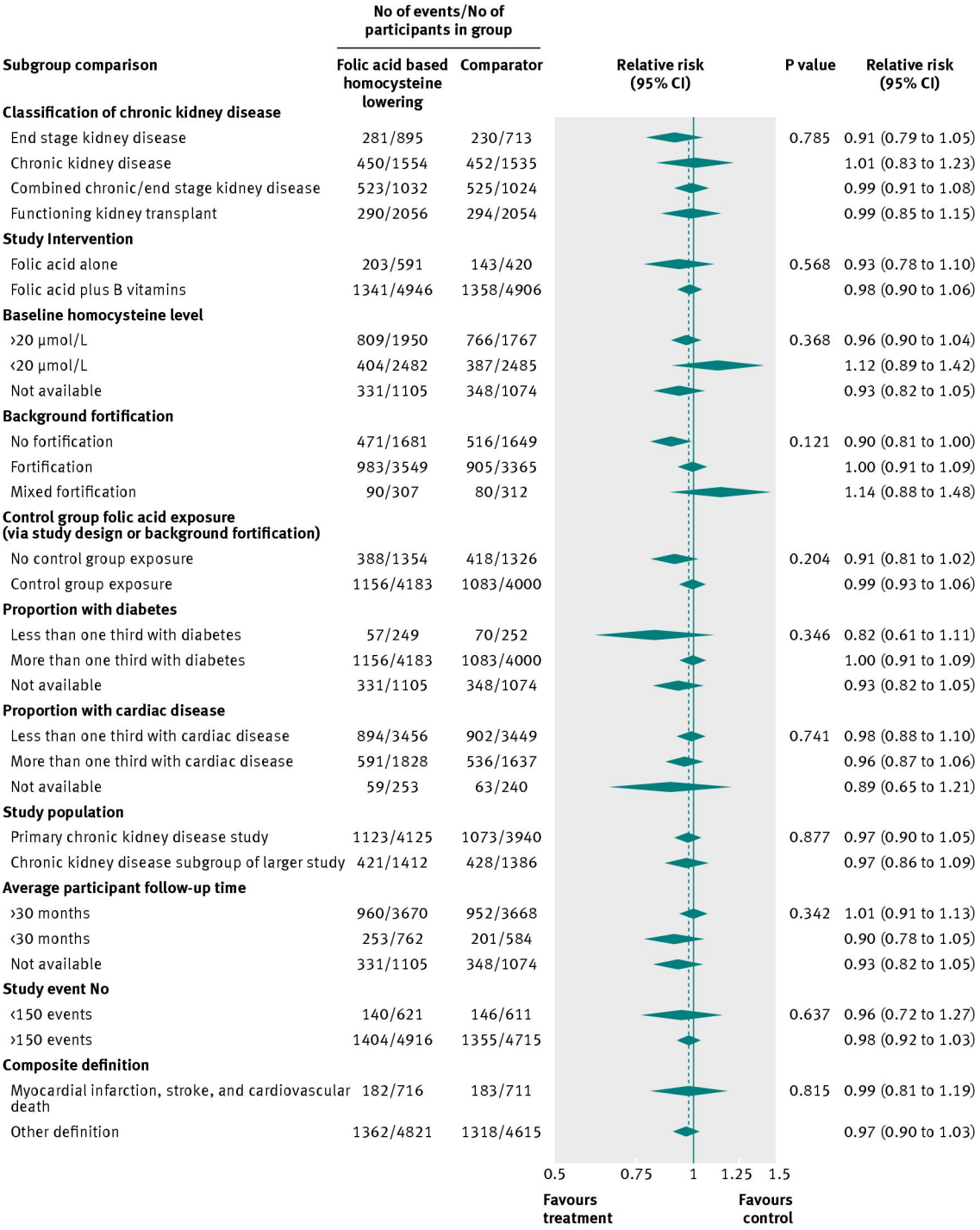

Fig 3 Subgroup analyses for effect of homocysteine lowering on cardiovascular events. Data reported as '"not available" applied to chronic kidney disease subgroups of relevant studies