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Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis

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Abstract

Objective To assess whether the association of serum homocysteine concentration with ischaemic heart disease, deep vein thrombosis and pulmonary embolism, and stroke is causal and, if so, to quantify the effect of homocysteine reduction in preventing them.

Design Meta-analyses of the above three diseases using (a) 72 studies in which the prevalence of a mutation in the MTHFR gene (which increases homocysteine) was determined in cases (n=16 849) and controls, and (b) 20 prospective studies (3820 participants) of serum homocysteine and disease risk. **Main outcome measures** Odds ratios of the three diseases for a 5 $\mu\text{mol/l}$ increase in serum homocysteine concentration.

Results There were significant associations between homocysteine and the three diseases. The odds ratios for a 5 $\mu\text{mol/l}$ increase in serum homocysteine were, for ischaemic heart disease, 1.42 (95% confidence interval 1.11 to 1.84) in the genetic studies and 1.32 (1.19 to 1.45) in the prospective studies; for deep vein thrombosis with or without pulmonary embolism, 1.60 (1.15 to 2.22) in the genetic studies (there were no prospective studies); and, for stroke, 1.65 (0.66 to 4.13) in the genetic studies and 1.59 (1.29 to 1.96) in the prospective studies.

Conclusions The genetic studies and the prospective studies do not share the same potential sources of error, but both yield similar highly significant results—strong evidence that the association between homocysteine and cardiovascular disease is causal. On this basis, lowering homocysteine concentrations by 3 $\mu\text{mol/l}$ from current levels (achievable by increasing folic acid intake) would reduce the risk of ischaemic heart disease by 16% (11% to 20%), deep vein thrombosis by 25% (8% to 38%), and stroke by 24% (15% to 33%).

Introduction

The serum concentration of the amino acid homocysteine is positively associated with the risk of ischaemic heart disease, deep vein thrombosis and pulmonary embolism, and stroke.^{1 2} There is uncertainty over whether these associations are causal.^{3 4} Resolving the question of causality is important because serum homocysteine can be lowered by the B vitamin folic acid,^{5 6} raising the prospect of a simple and safe means of prevention.

Large increases in serum homocysteine occur in homocystinuria, a rare autosomal recessive disorder that is associated with a high risk of premature cardiovascular disease.⁷ Moderate increases in serum homocysteine occur as a result of a mutation in the gene coding for the enzyme methylenetetrahydrofolate reductase (MTHFR) in which cytosine is replaced by thymidine (C→T). However, because the

increase in homocysteine is relatively small, studies comparing the risk of cardiovascular disease in people with and without the TT mutation need large numbers to be able to show any effect. Previous meta-analyses have had too few studies available to do this.

We present a new meta-analysis of the MTHFR studies (including data from an additional 34 studies since the previous meta-analyses),^{8–10} which has the statistical power to show an effect. We compare these results with those of a meta-analysis of prospective studies of serum homocysteine and disease events.

Methods

Identification of studies

We sought two types of study on the association between serum homocysteine and ischaemic heart disease, deep vein thrombosis with or without pulmonary embolism, or stroke:

- Studies reporting the prevalence of the genetic variant of the MTHFR enzyme (homozygous (TT) and heterozygous (CT) compared with “wild type” (CC)) in cardiovascular disease cases and controls
- Prospective (cohort) studies of serum homocysteine and disease events.

To identify studies, we searched databases (Medline, CINAHL, Embase, the *Cochrane Library*, PsycINFO, and ClinPSYC) in any language up to October 2001 (see bmj.com). We included 120 studies in total^(n1-w120) on bmj.com.

Statistical methods

For each MTHFR study we determined the odds ratio of being homozygous (TT), and in a separate analysis heterozygous (CT), for the mutant allele compared with being homozygous for the wild type allele (CC) in cases and controls.

In the prospective studies we estimated odds ratios for a 5 $\mu\text{mol/l}$ increase in serum homocysteine (a standard reference increment).

We used a random effects model to derive summary odds ratios from combinations of studies to allow for any heterogeneity across studies. In the prospective studies we adjusted the summary odds ratios for regression dilution bias¹¹; these arose because the serum homocysteine values in each person were from single measurements, which are unrepresentative of a person's long term average value over the period of follow up.

Results

Ischaemic heart disease

Studies of MTHFR mutation

The odds ratios of homozygotes for the mutant allele (TT) compared with wild type homozygotes (CC) are shown with the studies ranked in order of increasing effect in fig 1. There was a wide range of estimates, but

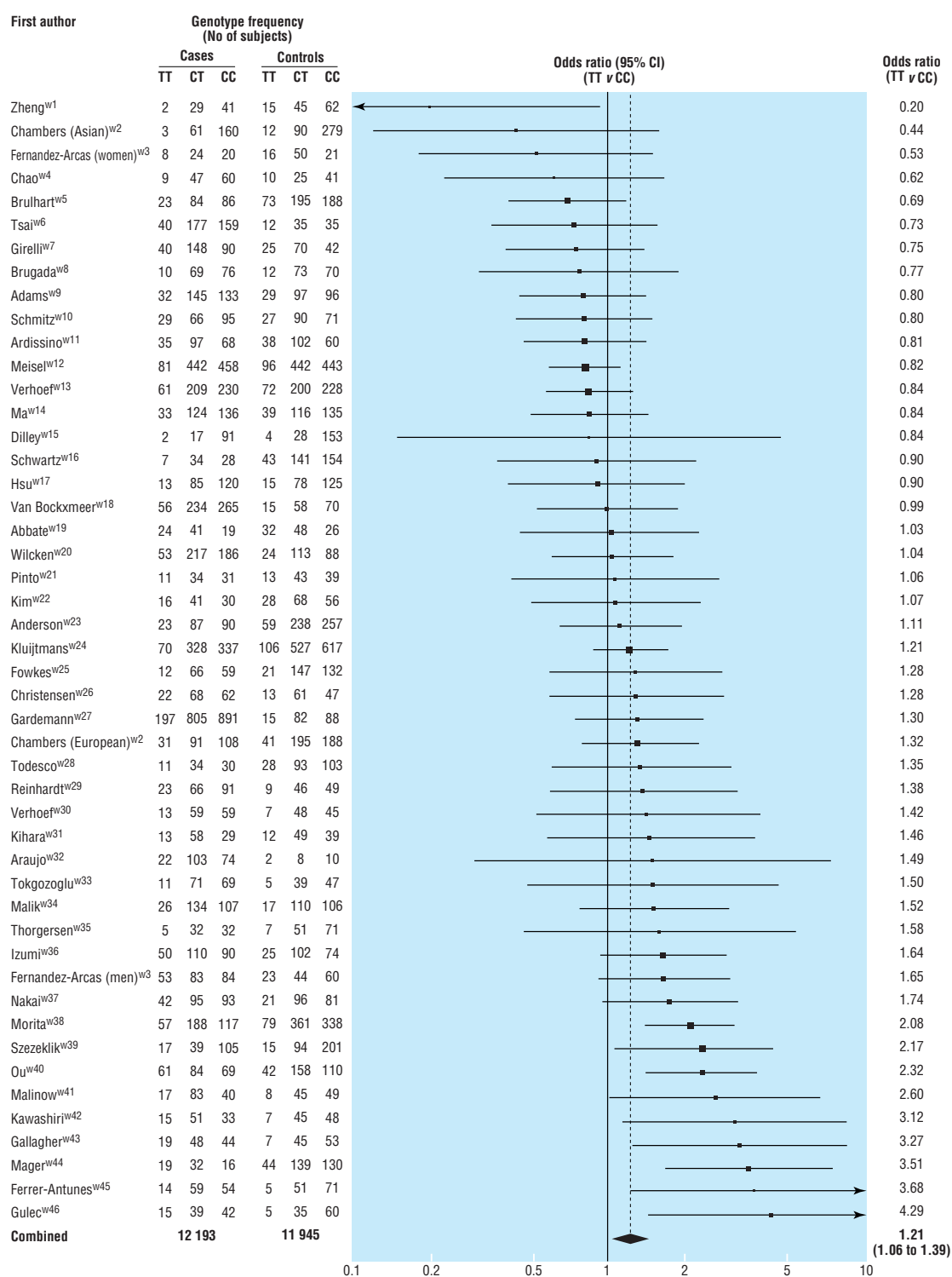


Fig 1 Results of published studies of association between MTHFR mutation and ischaemic heart disease: values are odds ratios (95% confidence intervals) for homozygotes for mutant allele (TT) v wild type (CC)

the summary odds ratio was 1.21 (95% confidence interval 1.06 to 1.39; $P=0.006$), indicating that risk was on average 21% higher in TT homozygotes than in CC homozygotes. The summary odds ratio for the heterozygous state (CT v CC) was 1.06 (0.99 to 1.13; $P=0.09$).

From the 33 studies that provided relevant data, the mean difference in serum homocysteine concentration

between the TT and CC genotypes was $2.7 \mu\text{mol/l}$ (2.1 to $3.4 \mu\text{mol/l}$) and that between the CT and CC genotypes was $0.29 \mu\text{mol/l}$ (0.20 to $0.39 \mu\text{mol/l}$). The summary odds ratio of 1.21 for TT v CC is equivalent to an odds ratio of 1.42 (1.11 to 1.84) for the standard $5 \mu\text{mol/l}$ increase in serum homocysteine (calculated by raising 1.21 to the power of $5/2.7$).

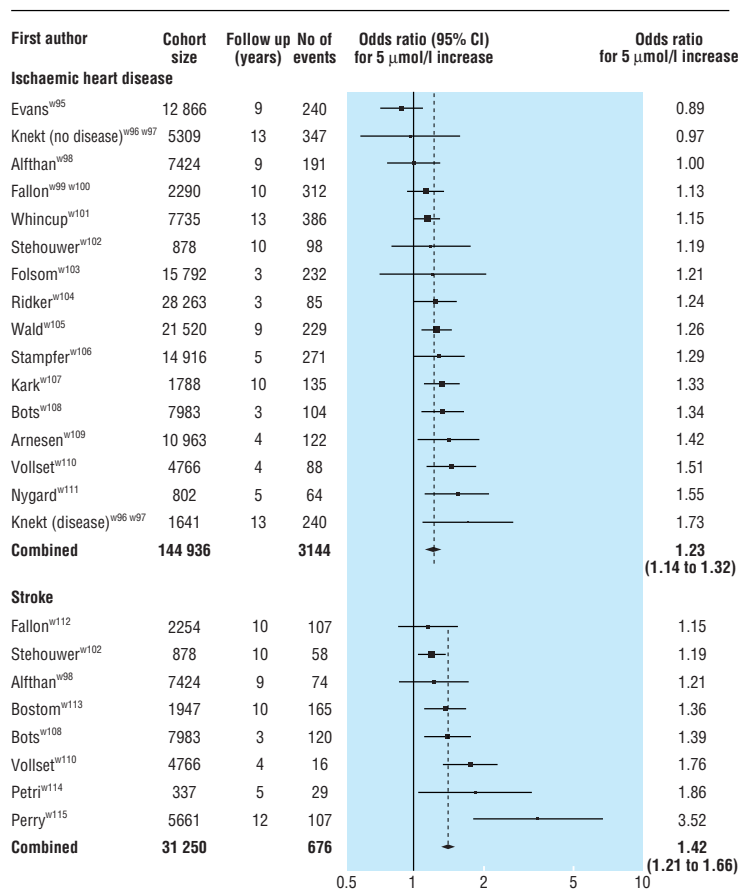


Fig 2 Results of prospective studies of serum homocysteine concentration and ischaemic heart disease and stroke: values are odds ratios (95% confidence intervals) for a 5 $\mu\text{mol/l}$ increase in serum homocysteine, adjusted for age, sex, smoking, cholesterol concentration, and blood pressure (except in one study, adjusted for age and sex alone^{w106}) but not for regression dilution bias

Combining the results from 12 of the MTHFR studies in which other cardiovascular risk factors were measured showed that TT and CC homozygotes had similar serum cholesterol concentration, blood pressure, smoking prevalence, and body mass index, with no significant differences. The upper confidence intervals of these risk factors' associations with TT homozygotes were 0.02 mmol/l, 3 mm Hg, 6%, and 0.9 kg/m² higher respectively; applying these to the quantitative association of each risk factor with ischaemic heart disease,¹² showed that even if there were confounding by another risk factor it could at most account for half the 22% excess risk seen in TT homozygotes.

Prospective studies of homocysteine and cardiovascular disease

The odds ratios are adjusted for age, sex, smoking, blood pressure, and serum cholesterol concentration in all the studies except one, which is adjusted for age and sex only. The summary odds ratio of 16 prospective studies on ischaemic heart disease was 1.23 (1.14 to 1.32) for a 5 $\mu\text{mol/l}$ increase in serum homocysteine concentration (fig 2), or 1.32 (1.19 to 1.45) adjusted for regression dilution bias. There was a suggestion of a trend across the prospective studies for a decreasing odds ratio with increasing age at the time

of the event (P=0.07), as has been observed for other cardiovascular risk factors (smoking, serum cholesterol, and blood pressure).

Deep vein thrombosis

Fig 3 shows the results of the 26 MTHFR studies of deep vein thrombosis (3439 cases, mean age at event 44 years). The summary odds ratio for homozygotes for the mutant allele (TT) compared with wild type homozygotes (CC) was 1.29 (1.08 to 1.54; P=0.007), equivalent to an odds ratio of 1.60 (1.15 to 2.22) for a 5 $\mu\text{mol/l}$ increase in serum homocysteine concentration. The summary odds ratio associated with the heterozygous state (CT v CC) was 1.05 (0.94 to 1.19; P=0.41).

There were no published prospective studies.

Stroke

The seven MTHFR studies of stroke (1217 cases, mean age at event 63 years) yielded relatively few data, so the confidence interval for the summary result was wide: the odds ratio for homozygotes for the mutation (TT) compared with wild type homozygotes was 1.31 (0.80 to 2.15), equivalent to an odds ratio of 1.65 (0.66 to 4.13) for a 5 $\mu\text{mol/l}$ increase in serum homocysteine. The summary odds ratio associated with the heterozygous state (CT v CC) was 1.15 (0.93 to 1.42).

The summary odds ratio of the eight prospective studies for a serum homocysteine increase of 5 $\mu\text{mol/l}$ was 1.42 (1.21 to 1.66) (fig 2), or 1.59 (1.29 to 1.96) adjusted for regression dilution bias. The results are adjusted for age, sex, smoking, blood pressure, and serum cholesterol in all the studies.

Discussion

The MTHFR studies show highly significant associations between serum homocysteine concentration and ischaemic heart disease and deep vein thrombosis. Previous meta-analyses of the MTHFR studies failed to show an effect because they lacked statistical power, but their confidence intervals were consistent with the significant estimates we present here.^{8-10 13} Our analyses combine data from an extra 23 studies on ischaemic heart disease and an extra 11 studies on deep vein thrombosis since the largest previous meta-analyses.^{9 13}

Interpretation of evidence for causality

The results of the MTHFR and the prospective studies can be explained in one of two ways—a direct (or causal) explanation or an indirect (non-causal) explanation. An indirect explanation would depend on the MTHFR and prospective studies both showing associations with homocysteine through confounding. In the MTHFR studies the difference in homocysteine concentration arises from a single gene mutation effectively allocated at random. There is therefore no basis for expecting that people with the mutant gene would systematically differ from those without it in other cardiovascular risk factors (smoking, blood pressure, serum cholesterol concentration), and the data show that they do not. Genetic confounding is theoretically possible, whereby a gene linked to the MTHFR gene controls some unknown risk factor and coincidentally increases serum homocysteine concentration.

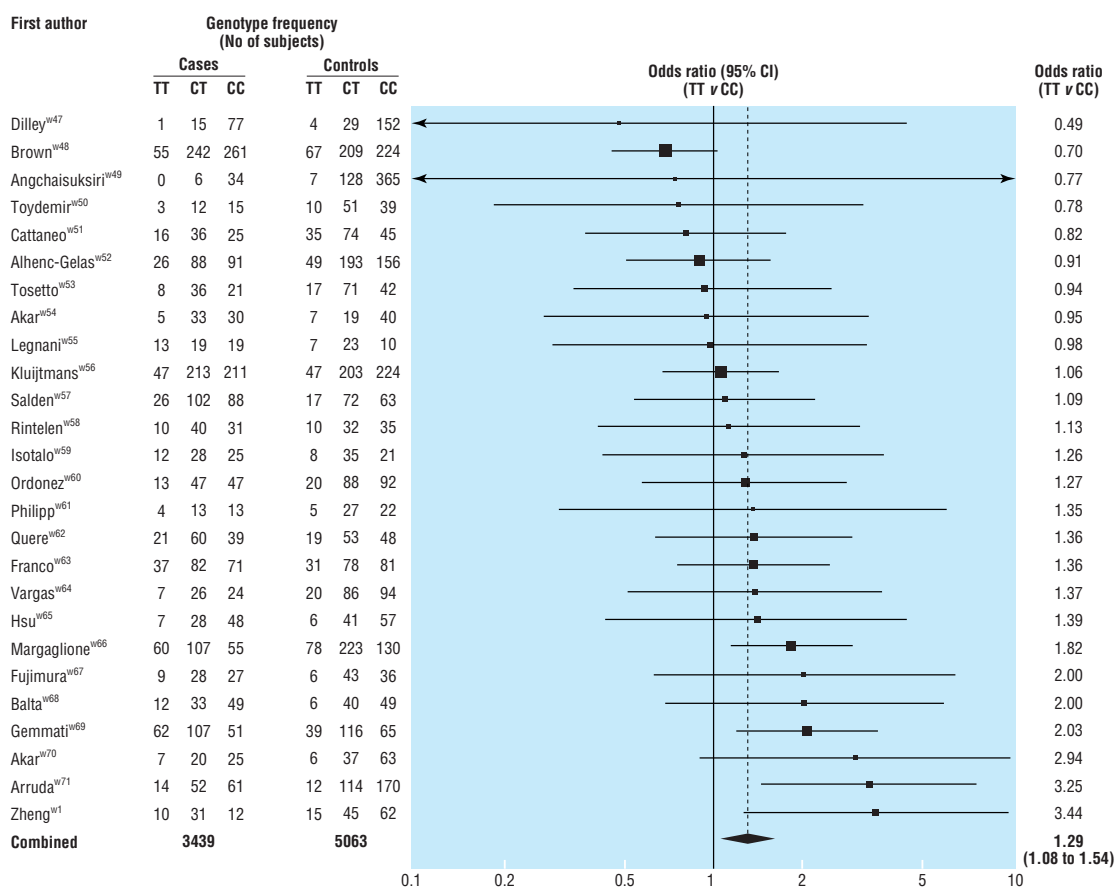


Fig 3 Results of published studies of association between the MTHFR mutation and deep vein thrombosis: values are odds ratios (95% confidence intervals) for homozygotes for mutant allele (TT) v wild type (CC)

In the prospective studies confounding from smoking, blood pressure, and serum cholesterol was allowed for; if there were any remaining confounding it would also be from an unknown factor associated with both serum homocysteine and cardiovascular disease. Importantly, the genetic linkage that one would have to postulate to explain the association with cardiovascular disease in the MTHFR studies could not account for the association in the prospective studies—because the increase in risk of about a quarter with the MTHFR variant is so much smaller than the twofold difference in risk (10th to 90th centile) in the prospective studies. The indirect explanation relies on a complex and

improbable series of different associations that coincidentally yield similar cardiovascular disease risks for a given difference in serum homocysteine concentration. The direct (causal) explanation is plausible and much simpler.

Substantial publication bias is unlikely because many of the individual study results were not statistically significant, and many of these were interpreted by their authors as being negative. A standard statistical assessment of publication bias (the regression asymmetry test¹⁴) showed no basis for concern in either the ischaemic heart disease studies (P=0.55) or the deep vein thrombosis studies (P=0.43).

Summary results from the MTHFR studies and the prospective studies on risk of ischaemic heart disease, deep vein thrombosis with or without pulmonary embolism, and stroke associated with serum homocysteine concentration

Study type	No of studies	No of cases	5 µmol/l increase in homocysteine		3 µmol/l decrease in homocysteine	
			Summary odds ratio (95% CI)	Combined odds ratio (95% CI)	Odds ratio (95% CI)	Risk reduction (95% CI)
Ischaemic heart disease:						
MTHFR	46	12 193	1.43 (1.11 to 1.84)	1.33 (1.22 to 1.46)	0.84 (0.80 to 0.89)	16% (11% to 20%)
Prospective*	16	3 144	1.32 (1.19 to 1.45)			
Deep vein thrombosis:						
MTHFR	26	3 439	1.60 (1.15 to 2.22)		0.75 (0.62 to 0.92)	25% (8% to 38%)
Stroke:						
MTHFR	7	1 217	1.65 (0.66 to 4.13)	1.59 (1.30 to 1.95)	0.76 (0.67 to 0.85)	24% (15% to 33%)
Prospective*	8	676	1.59 (1.29 to 1.96)			

*Prospective studies adjusted for regression dilution bias and for age, sex, blood pressure, and serum cholesterol concentration in all studies except one^{w106} (adjusted for only age and sex).

What is already known on this topic

There is an association between serum homocysteine concentration and cardiovascular disease, but it is not known whether the association is causal

A common single gene mutation that reduces the activity of an enzyme involved in folate metabolism (MTHFR) is associated with a moderate (20%) increase in serum homocysteine

What this study adds

A meta-analysis of MTHFR studies shows a significantly higher risk of both ischaemic heart disease and deep vein thrombosis (with or without pulmonary embolism) in people with the MTHFR mutation

A meta-analysis of prospective studies shows a significant association between homocysteine concentration and ischaemic heart disease similar in size to that expected from the results of the MTHFR studies and a significant association with stroke

The MTHFR studies and the prospective studies do not share the same potential sources of error but both yield similar results—strong evidence that the association between homocysteine and cardiovascular disease is causal

On this basis a decrease in serum homocysteine of 3 $\mu\text{mol/l}$ (achievable by daily intake of about 0.8 mg folic acid) should reduce the risk of ischaemic heart disease by 16%, deep vein thrombosis by 25%, and stroke by 24%

Evidence of risk reduction

A placebo controlled randomised trial of treatment with B vitamins (folic acid, B-6, and B-12) to lower serum homocysteine concentration in patients with ischaemic heart disease has shown a rapid reduction of risk.¹⁵ Studies of patients with homocystinuria treated with B vitamins have also shown reduction in risk.⁷⁻¹⁶

The table summarises the odds ratios of ischaemic heart disease, deep vein thrombosis, and stroke for the standard 5 $\mu\text{mol/l}$ increase in homocysteine concentration and shows combined odds ratios from the two types of study. For ischaemic heart disease this was 1.33 (95% confidence interval 1.22 to 1.46), and for stroke it was 1.59 (1.30 to 1.95). The table also converts the odds ratios for a 5 $\mu\text{mol/l}$ increase in homocysteine concentration into odds ratios for a 3 $\mu\text{mol/l}$ decrease in homocysteine (the maximal effect of folic acid, achieved with a daily dose of about 0.8 mg).⁵⁻⁶ On the basis that the association is causal and reversible, we estimate that folic acid could reduce the risk of ischaemic heart disease by 16%, deep vein thrombosis by 25%, and stroke by 24%. The folic acid could be taken as tablets by high risk patients, and possibly supplied to the general public through food fortification or a combination of both.

Conclusion

Our results strengthen the evidence that a raised serum homocysteine concentration is a cause of cardiovascular disease. Our conclusion rests on the following observations. (1) The genetic (MTHFR) studies show a moderate increase in risk for a moderate increase in serum homocysteine; genetic linkage to some unknown risk factor might be the explanation, although no such linkage is known. (2) The prospective studies show an association between serum homocysteine and cardiovascular disease after allowance for confounding. (3) These two types of study are susceptible to different sources of error but show quantitatively similar associations, a result that is unlikely to have occurred through different potential sources of confounding acting independently. (4) The homocystinurias cause high serum homocysteine levels and high risks of premature cardiovascular disease. (5) Lowering serum homocysteine reduced risk, both in a randomised trial in patients with heart disease and in patients with homocystinuria.

In the light of these five observations we could not have concluded otherwise.

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