Homocysteine and cardiovascular disease: a review of the evidence

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Abstract

Elevated homocysteine (HCY) levels can be caused by a number of factors, including folate and B-vitamin deficiency, pre-existing atherosclerotic disease, diabetes and various drugs. Epidemiological evidence, as well as data from retrospective and prospective studies, supports an association between elevated HCY levels and increased risk of cardiovascular disease (CVD). However, whether lowering HCY levels by administration of folate and vitamins B6 and B12 is associated with any significant decrease in vascular risk remains the subject of ongoing debate. Although the major studies that have reported to date show that vitamin supplementation was associated with a decrease in HCY levels, this failed to have any significant effect on cardiovascular risk. Furthermore, although some lipid-modifying treatments have been shown to increase HCY levels, there is no evidence that this attenuates or compromises the beneficial effects of such treatments on cardiovascular risk.

Taken together, these data suggest that HCY is a marker, rather than a cause, of CVD and therefore do not provide support for routine screening for and treatment of elevated HCY to prevent CVD. Data from ongoing clinical trials are awaited to clarify this issue.

Diabetes Vasc Dis Res 2007;4:143–9
doi:10.3132/dvdr.2007.033

Key words: cardiovascular risk, homocysteine, lipid-modifying therapy, vitamin supplementation.

Introduction

Epidemiological studies long ago identified age, gender, smoking, lipids, hypertension and diabetes along with psychological factors, lack of exercise and a low fruit and vegetable diet as cardiovascular disease (CVD) risk factors. Among the dietary risk factors, inadequate vitamin intakes have long been suspected to be CVD risk factors. While antioxidant vitamins have failed to show benefits, other vitamins still remain targets for intervention. A number of populations at high CVD risk, including Celts and Indo-Asians, have regularly been shown to have high rates of folate and cyanocobalamin (vitamin B12) deficiencies, associated with increased levels of an intermediate in one carbon metabolism – homocysteine (HCY).

HCY is a sulphur-containing amino acid in the body produced by conversion of methionine, an essential amino acid present in foods regularly consumed within the diet. Low levels of HCY (5–15 μmol/L) are normally found in the plasma. However, genetic defects in the enzymes of HCY metabolism markedly increase HCY levels. Mutations in cystathionine-β-synthase are associated with huge excess levels of HCY (> 100 μmol/L) and an excess rate of premature thrombotic events, including stroke and venous thrombosis. Although homocystinuria is rare, mild elevations of HCY (plasma concentrations of 15–25 μmol/L) are common in many populations. These slight elevations are associated with diet and also with common single nucleotide polymorphisms in enzymes in the HCY metabolic pathway. However, to understand the potential relevance of HCY to CVD, it is important first briefly to review pathways involved in HCY metabolism.

Homocysteine metabolism

HCY is metabolised via two major pathways (figure 1). When there is excess methionine, HCY is metabolised via the trans-sulphuration pathway, resulting in the production of cystathionine, a process requiring vitamin B6 as a cofactor, and subsequently to cysteine. Any excess cysteine is oxidised to taurine or sulphates or eliminated from the body. However, when methionine levels are low, HCY is mainly metabolised via a methionine-conserving pathway. In most
tissues, this involves remethylation of HCY to methionine, a process requiring methyltetrahydrofolate (from folic acid) and vitamin B_{12} as cofactors. These two pathways are coordinated by S-adenosylmethionine (SAM), which is the sole source of methyl groups for all methylation reactions within the cell. S-adenosylhomocysteine (SAH), the by-product of these methylation reactions, is rehydrolysed thereby regenerating HCY, which is then available to start a new cycle of methyl-group transfer (figure 1). Thus, high levels of HCY are associated with reduced methylation potential, whereas folate and vitamin B_{12} increase this potential. Changes in the concentration of methionine in the body, particularly as a result of dietary intake of methionine, affect the rate of SAM synthesis, as well as the metabolism of HCY.

**Epidemiological evidence**

Studies in animal models have shown that elevated HCY levels result in increased oxidant stress, impaired endothelial function, and increased thrombogenicity, which act together to promote atherosclerosis. Although cross-sectional and case-control studies have indicated an association between plasma concentrations of HCY and the extent of carotid, coronary and peripheral vascular disease, it should be emphasised that the variables measured in these studies are only surrogate measures of CVD.

**Retrospective studies**

In a meta-analysis of 27 observational studies including about 4,000 subjects, hyperhomocysteinaemia (defined as plasma HCY levels greater than the 90th or 95th percentile of levels in controls) was associated with an increased risk of atherosclerotic disease. Analysis suggested that an increase in basal total plasma HCY levels of 5 μmol/L was associated with 60% and 80% increased risk of CHD in men and women, respectively, similar to the effect of raising cholesterol by 0.5 mmol/L. Subsequent observational studies have provided consistent support for an association between hyperhomocysteinaemia and atherosclerotic vascular disease. The largest of these, the European Concerted Action Project, which included 3,350 men and women with arterial vascular disease and 1,000 controls, showed that an increase in plasma HCY levels was an independent risk factor for CVD. Subjects with total HCY levels > 80th percentile had a 2.2-fold (95% CI 1.6 to 2.9) increased risk for CVD compared with those with HCY levels ≤ 80th percentile.

**Prospective studies**

Findings from prospective cohort studies that evaluated the association between an increase in HCY levels and CVD have been inconsistent. Some of these studies reported a statistically significant positive association between elevated HCY and coronary heart disease (CHD) and stroke (table 1). In contrast, other studies failed to demonstrate a significant association between plasma HCY and CHD. Data from the Physicians’ Health Study, a nested case-control study including 333 male patients and 333 controls (from a total population of 14,916 male patients) followed up for a mean of 7.5 years, failed to demonstrate any significant association between elevated HCY and risk for myocardial infarction (MI) and CHD death (relative risk 1.7, 95% CI 0.9 to 3.3, for subjects with ≥ 95th percentile vs. < 95th percentile of total HCY levels). Additionally, the Multiple Risk Factor Intervention Trial cohort, the Atherosclerosis Risk in Communities Study cohort and the North Karelia Project failed to show any significant association between elevated HCY levels and risk of major coronary events or stroke (table 1). Prolonged follow-up from the Physicians’ Health Study also demonstrated a lack of association between plasma HCY levels and risk for stroke and angina. It is possible, however, that the HCY-lowering effect of folate-fortified flour may have been a confounder in these later studies.

Subsequent meta-analysis of prospective observational studies of first events demonstrated an association between hyperhomocysteinaemia and elevated risk of CVD. An increase in plasma HCY levels by 25% (i.e. about 3 μmol/L) was associated with 11% and 19% excess risk for ischaemic heart disease (IHD) and stroke, respectively, after correction for other cardiovascular risk factors. However, bias may have existed in this analysis, as the relative risks associated with elevated HCY by 3 μmol/L were 1.49 (95% CI 1.41 to 1.61) for IHD and 1.16 (95% CI 0.99 to 1.37) for cerebrovascular accident (CVA) in retrospective studies, but 1.20 (95% CI 1.12 to 1.28) for IHD and 1.30 (95% CI 1.11 to 1.52) for CVA in prospective studies. Furthermore, the numbers of strokes in these studies were relatively small. Similar results were obtained in a retrospective analysis of 16,840 patients for a 5 μmol/L increase in HCY.

Given the definite functional nature of the methylene-tetrahydrofolate reductase (MTHFR) single nucleotide polymorphism and its relationship to plasma HCY levels, it is possible to perform Mendelian randomisation analyses of cohort studies. In a meta-analysis by Wald et al., comparison of the high risk TT genotype with other genotypes showed a 21% (95% CI 6% to 39%) increased risk of IHD and a non-significant 31% (95% CI -20% to +215%) increased risk of stroke. A subsequent meta-analysis of 15,635 cases using Mendelian randomisation showed that a 1.93 (range 1.38–2.47) μmol/L increase in HCY was associated with a 1.26 (95% CI 1.14 to 1.40)-fold increase in CHD death and 1.26 (95% CI 1.14 to 1.40)-fold increase in CVA risk, close to the 1.20 (95% CI 1.13 to 1.30)-fold increase in risk predicted on plasma levels alone.

Taken together, evidence from case-control studies as well as prospective studies supports an association between elevated plasma HCY levels and increased cardiovascular risk. However, whether lowering HCY levels by administration of folate and vitamins B_{6} and B_{12} is associated with any significant decrease in vascular events in populations at risk remains the subject of ongoing debate.

**Evidence from clinical trials**

Of a number of large prospective studies initiated to address this issue, involving a projected total of 52,000 subjects, there have recently been reported. In the first of these, the Vitamin Intervention for Stroke Prevention (VISP) study, 3,680 patients who had had a recent stroke were randomly assigned to treatment with folic acid, vitamin B_{12} and vitamin B_{6} at either high or low doses. Although there was evidence of a dose-dependent reduction in HCY, there was no
significant difference between the two groups in the rates of stroke (the primary end point) or a composite of vascular outcomes (recurrent stroke, CHD event or death) at the end of the two-year follow-up period (table 2). However, this trial was limited by its low dose-high dose design, recruitment in the US (where flour is folate-fortified), vitamin B12 pre-treatment, and low rates of stroke. A post hoc analysis that excluded those patients with low or very high B12 levels or with significant renal dysfunction did, however, indicate a 21% benefit on major cardiovascular events (p=0.049; adjusted for confounders p=0.056) associated with vitamin B12 treatment.32

The findings of two subsequent studies, the Norwegian Vitamin (NORVIT) trial33 and the Heart Outcomes Prevention Evaluation (HOPE) 2 study,34 were consistent with those of VISP. NORVIT was a secondary prevention trial including 3,749 men and women with prior MI who were randomly assigned to one of four treatments administered once daily: folic acid, vitamin B6 and vitamin B12 (group A); folic acid and vitamin B12 (group B); vitamin B6 alone (group C); or placebo on top of optimal cardiovascular drug care (group D). After a median follow-up of 40 months, combination vitamin treatment lowered mean total HCY levels by 27% and increased folate levels by

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Major end points</th>
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<th>Relative risk (95% CI)</th>
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<tbody>
<tr>
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<td>Cases</td>
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<tr>
<td>Physicikers’ Health Study</td>
<td>271 male cases + 271 controls</td>
<td>Fatal and non-fatal MI, CHD death</td>
<td>5</td>
<td>11.1</td>
<td>10.5</td>
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<tr>
<td>Stamper et al.13</td>
<td>333 male cases + 333 controls</td>
<td>Fatal and non-fatal MI, CHD death</td>
<td>7.5</td>
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<tr>
<td>Arnesen et al.12</td>
<td>123 male and female cases + 492 controls</td>
<td>Fatal and non-fatal CHD</td>
<td>3.5</td>
<td>12.7</td>
<td>11.3</td>
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<tr>
<td>British Regional Heart Study</td>
<td>107 male cases + 118 controls</td>
<td>Fatal and non-fatal stroke</td>
<td>12.8</td>
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<tr>
<td>Perry et al.16</td>
<td>229 male cases + 1,126 controls</td>
<td>Fatal CHD</td>
<td>8.7</td>
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</tr>
<tr>
<td>Rotterdam Study</td>
<td>224 male and female cases + 533 controls</td>
<td>Stroke and MI</td>
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<td>Stroke: 14.4</td>
<td>Mi: 17.2</td>
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<td>Stroke:</td>
<td>14.4</td>
<td>Mi: 17.2</td>
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<tr>
<td>ARIC</td>
<td>232 male and female cases + 537 controls</td>
<td>All CHD events</td>
<td>3.3</td>
<td>8.9</td>
<td>8.5</td>
</tr>
<tr>
<td>Folsom et al.19</td>
<td>93 male patients with MI + 186 controls; 147 male patients who died from CHD + 266 controls</td>
<td>Non-fatal MI, CHD death</td>
<td>14–17</td>
<td>12.6</td>
<td>13.1</td>
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<tr>
<td>MRFIT</td>
<td>Evans et al.18</td>
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<td>Men: 10.0</td>
<td>Women: 9.6</td>
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<td></td>
<td></td>
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<td>6.9</td>
<td>Women:</td>
<td>9.2</td>
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<tr>
<td>North Karelia Project</td>
<td>265 male and female cases + 249 controls</td>
<td>Fatal and non-fatal MI, stroke</td>
<td>9</td>
<td>Men: 10.0</td>
<td>Women: 9.6</td>
</tr>
</tbody>
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Key: MRFIT = Multiple Risk Factor Intervention Trial; ARIC = Atherosclerosis Risk in Communities study; CHD = coronary heart disease; MI = myocardial infarction; * statistically significant; CVD = cardiovascular disease

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600–700% in patients receiving folic acid plus vitamin B6, but had no significant effect on the primary end point (a composite of recurrent MI, stroke and sudden death due to CHD). Event rates for the primary end point were 18% in groups B to D. In the triple therapy group (group A) the event rate was increased by 22% (95% CI 0 to 50%, p=0.05) and for non-fatal MI by 50% (p=0.05), countered by a non-significant 17% decrease (p=0.52) in stroke. Overall, the event rate for the primary end point with triple therapy (group A) compared with the other groups was increased by 20% (95% CI 2% to 41%) (table 2). Some analyses of vitamin B6 trial data have suggested that high-dose vitamin B6 therapy may increase cardiovascular events. In NORVIT a 14% increase in events (p=0.09) was noted with vitamin therapy. A Bayesian analysis of vitamin therapy using data from the NORVIT and HOPE 2 studies suggested that there is little effect of supplements on the rates of cardiovascular events, mortality or MI although there may be a beneficial effect on rate of stroke.31

In a smaller study (n=205), treatment with the combination of folic acid, vitamin B6 and vitamin B12 for six months was shown to reduce significantly the rate of restenosis (19.6% vs. 37.6% on placebo, p=0.01) and the need for revascularisation of the target lesion (10.8% vs. 22.3% on placebo, p<0.05) after coronary angioplasty.32 In an extension of this study, including 553 subjects who had undergone successful angioplasty of at least one significant stenosis, vitamin treatment was associated with a significant decrease in the incidence of the composite end point of major adverse events (i.e. death, non-fatal MI and need for repeat revascularisation) after a mean follow-up of 11 months (relative risk 0.68, 95% CI 0.48 to 0.96, p=0.03).33 Another study showed, however, that vitamin treatment might increase the rate of stenosis after coronary stenting.34

Most recently, a meta-analysis of 12 randomised controlled studies of folic acid supplementation, including data from 16,958 subjects with pre-existing vascular disease,
Table 3. Common causes of homocysteinuria

- Age
- Male sex
- Menopause
- Lifestyle factors: smoking, coffee intake
- Vitamin deficiencies: folic acid, vitamin B6 and vitamin B12
- Hepatic dysfunction
- Renal dysfunction
- Diabetes mellitus
- Malignancies
- Drugs: folate antagonists, vitamin B12 antagonists, anticonvulsants, metformin, thiazide diuretics, some glitazones, some lipid-modifying treatments (colestipol, nicotinic acid, fibrates)

showed that folic acid supplementation did not significantly reduce cardiovascular risk or all-cause mortality. The overall relative risks for subjects treated with folic acid supplementation compared with controls were 0.95 (95% CI 0.88 to 1.03) for CVD, 1.04 (95% CI 0.92 to 1.17) for CHD, 0.86 (95% CI 0.71 to 1.04) for stroke and 0.96 (95% CI 0.88 to 1.04) for all-cause mortality.43

The disparity between evidence from epidemiological and retrospective and prospective case-control studies and the results of these recent clinical trials could be due to inherent limitations in the observational studies. A wide range of conditions are known to increase plasma HCY levels, including renal and hepatic impairment, diabetes and hypertriglyceridaemia;40 in addition, other cardiovascular risk factors such as smoking and elevated blood pressure are also associated with increased HCY levels42 (table 3). Furthermore, individuals with pre-existing atherosclerosis have higher HCY levels than those without. Thus, it has been suggested by the HOPE2 investigators that HCY is a marker, rather than a cause, of vascular disease, and therefore epidemiological data could be the result of residual confounders. Given the conflicting results to date, further trial evidence is required. The Western Norway B-vitamin Intervention Trial (WENBIT), Study of the Effectiveness of Additional Reduction in Cholesterol and Homocysteine (SEARCH), Prevention with A Combined Inhibitor and Folate In Coronary heart disease (PACIFIC) and VITamins TO (SEARCH), Prevention with A Combined Inhibitor and Folate Additional Reduction in Cholesterol and Homocysteine Intervention Trial (WENBIT), Study of the Effectiveness of founders. Given the confusing results to date, further trial epidemiological data could be the result of residual concentrations.43 In contrast, statins have no effect on plasma HCY transfer.43

The most likely mechanism for this increase is an alteration of creatine–creatinine metabolism and changes in methyl transfer.41 In contrast, statins have no effect on plasma HCY concentrations.43

In addition, other agents commonly prescribed in patients with CVD affect HCY levels. Thiazide diuretics are associated with rises in creatinine and a 16% increase in plasma HCY.46 An HCY-raising effect of metformin has been known since 1971, associated with a deficiency in vitamin B12 due to reduced uptake.47 Studies have shown that metformin reduces vitamin B12 levels by 10–12% and folate by 8% and raises HCY by 13%.48 The effects of metformin on HCY levels can be ameliorated through the use of calcium supplements.49 More recently, 20% increases in HCY (10.7 μmol/L; p<0.001) have been described with rosiglitazone50 while sulphonylureas have been shown to decrease HCY.4 Combining metformin and glitazones are associated with varying effects with reduction in HCY seen with pioglitazone compared with rosiglitazone.50-51 Antacids are also associated with reductions in acid-induced cobalamin release from food and hence secondary decreases in absorption.

Both fenofibrate and bezafibrate have been shown to induce elevation in plasma levels of HCY.41 In a direct comparative study, in which patients were randomised to treatment with fenofibrate or atorvastatin for six months (after an initial six-week placebo run-in period), fenofibrate induced a significant 35% increase in HCY levels (from 12.3 [3.9] μmol/L to 16.4 [4.6] μmol/L; p<0.0001), whereas there was no significant change in the group receiving atorvastatin.52 More recently, elevated plasma total HCY levels associated with treatment with fenofibrate were noted in both the Diabetes Atherosclerosis Intervention Study (DAIS)53,54 and the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial.55

In DAIS, in 418 patients with type 2 diabetes, treatment with fenofibrate 200 mg/day was associated with a 55% increase in plasma total HCY levels (from 11.5 [5.6] to 16.5 [10.7] μmol/L; p<0.001). This increase was not related to changes in factors known to modulate plasma HCY levels, including serum levels of vitamin B12 and folate, or renal dysfunction. Subsequent analysis showed that baseline, but not end-of-study, elevated plasma HCY levels decreased the beneficial effect of fenofibrate on angiographic determinants of focal coronary artery disease. Furthermore, HCY levels at the end of the study correlated negatively with coronary artery disease progression when data from all study patients were included in the analysis. In the fenofibrate group, there was no significant correlation between plasma total HCY levels and minimal lumen diameter, percent stenosis or adverse clinical events. Thus, the DAIS Investigators concluded that the fenofibrate-mediated increase in plasma total HCY levels observed did not attenuate the beneficial effects of fenofibrate on coronary artery disease or clinical events.54

FIELD included 9,795 patients with type 2 diabetes (78% without prior cardiovascular disease) who were randomised to treatment with fenofibrate 200 mg/day or placebo following a 16-week run-in period, comprising four weeks of dietary modification, six weeks of single-blind placebo and six weeks of single-blind fenofibrate therapy. The mean duration of follow-up in the study was five years. At the end of the study, plasma HCY levels were on average 35% higher in the fenofibrate group than the placebo group (median concentrations 15.1 μmol/L vs. 11.2 μmol/L, p<0.001).
However, in a subset of fenofibrate-treated patients who were restudied after study completion, plasma HCY levels fell from a median of 15.0 μmol/L to 9.5 μmol/L, indicating that the effect of treatment was reversible.31 This effect remains the subject of ongoing subgroup analyses by the FIELD Management Committee.

Taken together, these findings indicate that although fenofibrate does appear to increase plasma total HCY levels, this effect does not appear to attenuate or compromise the beneficial effects of treatment, and is reversible following discontinuation of therapy. Interactions between hypoglycaemic agents and lipid-lowering drugs in diabetes are likely to be complex and related to baseline renal function, autoimmune status, other drugs and supplements, as well as drugs used in the management of atherosclerosis.

Conclusions

Epidemiological evidence and data from observational studies support an association between elevated HCY levels and increased risk of CVD. However, whether lowering HCY levels by administration of folic and vitamins B6 and B12 is associated with any significant decrease in vascular events in populations at risk remains the subject of ongoing investigation. Of a number of large prospective studies initiated to address this issue, the three major studies that have reported to date have failed to show any significant effect of vitamin supplementation on cardiovascular risk; in fact, one of these studies (NORVIT) showed a trend for increased risk among patients who received folic acid plus vitamins B6 and B12.33

Plasma HCY levels are increased by a wide range of factors (table 3); as well, individuals with pre-existing atherosclerosis have higher HCY levels than those without. These factors may have confounded the results of epidemiological studies. In addition, certain drugs may also increase HCY levels. Fibric acid, nicotinic acid and colestipol have been shown to increase HCY levels, suggesting the potential for attenuation of clinical benefit. However, evidence from key fibrate studies such as FIELD and FIELD failed to show any compromise in the beneficial effects of fenofibrate on CVD prevention,55 which may be attributable to elevated HCY levels. Moreover, FIELD also showed that this effect on HCY was reversible following discontinuation of treatment.31

In conclusion, epidemiological observations of an association between elevated HCY levels and cardiovascular risk do not prove the existence of a causal relation, as they may be subject to a number of confounders. Furthermore, clinical trials such as NORVIT, VISP and HOPE 21,13,18 showed that, even though vitamin supplementation reduced HCY levels, there was no significant effect on cardiovascular risk. This is consistent with a recent meta-analysis which showed that folic acid supplementation was ineffective as a secondary prevention strategy for CVD.31 Taken together, these data suggest that HCY is a marker rather than a cause of CVD and therefore do not provide support for routine screening for and treatment of elevated HCY to prevent CVD.31 Data from ongoing studies are awaited to clarify this issue further.

Acknowledgements

The preparation of this review was funded by an unrestricted educational grant from Solvay Pharmaceuticals.

Conflict of interest declaration

ASW has received honoraria for lectures and advisory boards as well as travel and research grants from Astra-Zeneca, Bristol-Myers-Squibb, Genzyme, GlaxoSmithKline, LifeCycle Pharma, Merck kGA, Merck, Sharp & Dohme, Novartis, Pfizer, Sanofi-Aventis, Schering-Plough, Solvay-Fournier and Takeda.

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