The role of homocysteine in the clinical assessment of cardiovascular risk

JENNIFER BEXLEY

Abstract

Clinical and epidemiological studies suggest elevated levels of total plasma homocysteine (> 15 µmol/L) are associated with an increased risk of cardiovascular disease, independent of other known risk factors. This review outlines the causes of hyperhomocysteinaemia, current evidence of a positive association with cardiovascular disease, and how such findings may have important implications for future assessments of risk and nutritional recommendations, particularly for those with a previous or family history of cardiovascular disease.

Key words: homocysteine, cardiovascular disease, cardiovascular risk, vitamin supplementation.

Br J Cardiol 2003;**10**:118–22

Introduction

Disorders of the heart and circulation continue to be significant global health and economic issues, especially in industrialised societies. In Britain, it is estimated there were over 235,000 cardiovascular-related deaths in 2000 alone. This represents a sizeable proportion of health service resources with an estimated annual cost of over £1,600 million. It is pertinent to note that only 1% of this cost was spent on prevention. Such is the scale of the problem, the UK Government set a target for 2010 to reduce cardiovascular mortality in individuals under 75 years of age by at least two fifths.

Since a number of cardiovascular deaths may be preventable, identification of at-risk individuals and primary prevention is of paramount importance. In the search for novel cardiovascular risk factors, a strong positive association between plasma homocysteine and cardiovascular disease has emerged. Increasing epidemiological evidence now suggests that high homocysteine levels may be a significant independent cardiovascular risk factor.

The underlying atherogenic mechanisms of homocysteine remain unclear, although several biologically plausible mechanisms have been presented. These have implicated high levels of homocysteine with oxidation of low density lipoprotein (LDL) cholesterol via the generation of reactive oxygen species,[2] endothelial dysfunction,[3,4] proliferation of vascular smooth muscle cells,[5] and activation of platelet and coagulation factor.[4,6]

York Nutritional Laboratories, Murton Way, Osbaldwick, York, YO19 5US.
Jennifer Bexley, Project Scientist
Correspondence to: Mrs J Bexley
(email: jenniferb@yorktest.com)

Overview of homocysteine metabolism

Homocysteine is a sulphur-containing amino acid biosynthesised during metabolism of the essential amino acid methionine. Circulating levels of homocysteine are normally low due to its rapid metabolism via one of two pathways: a cobalamin (vitamin B_{12}) and folate dependent re-methylation pathway that regenerates methionine, or a pyridoxal 5′ phosphate (PLP; vitamin B_6) dependent trans-sulphuration pathway that converts homocysteine into cysteine (figure 1).

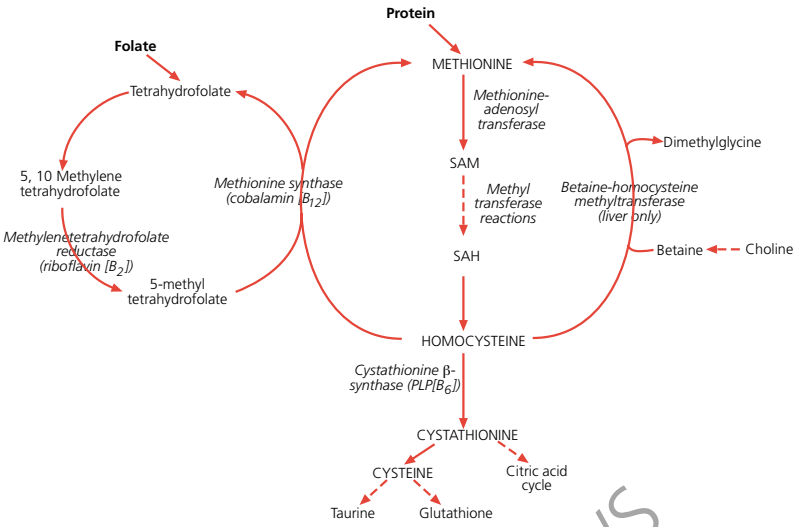
Homocysteine and cardiovascular disease

The relationship between high homocysteine levels and cardiovascular disease was first recognised over 30 years ago when it was observed that individuals with the rare condition homocysteinuria were predisposed to the development of premature atherosclerosis despite the absence of established cardiovascular risk factors. Homocysteinuria is a hereditary inborn error of methionine metabolism characterised by abnormally high levels of homocysteine in the blood and urine. It can arise from a deficiency in one of three fundamental enzymes in homocysteine metabolism: cystathionine β-synthase, methionine synthase, or methylenetetrahydrofolate reductase (MTHFR).

Studies of twins,[7,8] family members,[9-11] and reports of an association between hyperhomocysteinaemia and early familial coronary artery disease[12] suggest genetic factors play a key role in determining homocysteine levels, and may partly explain the familial tendency of cardiovascular disease. Numerous epidemiological studies have found higher levels of blood homocysteine in patients with heart disease, stroke, peripheral vascular disease and thromboembolic disease compared with age-matched controls.[13-15] Evidence of a positive association has been supported by a meta-analysis of retrospective data, which estimated that 10% of all cases of cardiovascular disease could be attributed to elevated concentrations of plasma homocysteine.[16] Furthermore, a rise in serum homocysteine of 5 µmol/L was sufficient to increase cardiovascular risk by 20–30%.[17] Homocysteine may be associated with other known risk factors and appears to confer a substantially increased risk in smokers, patients with hypertension, and those with elevated cholesterol.[18]

A number of prospective studies have observed a strong rela-

Figure 1. Metabolism of homocysteine



Key: SAM = S-adenosylmethionine; SAH = S-adenosylhomocysteine. An alternative re-methylation pathway exists in the liver, which utilises betaine as a methyl donor

tion between increasing levels of homocysteine and the onset of an initial cardiovascular event.[19-22] More recently, meta-analyses were performed on 72 genetic studies (16,849 cases with mutations in the MTHFR gene plus controls) and 20 prospective studies of serum homocysteine and disease risk (3,820 participants). Despite being subject to different sources of error, both the genetic and the prospective studies showed a significant positive association between homocysteine and cardiovascular disease, providing compelling evidence of a causal relationship.[23]

Homocysteine may also be associated with secondary cardiovascular events; a prospective study on over 500 patients with notable stenosis on coronary angiography observed a dose-response relation between baseline homocysteine levels and mortality from coronary artery disease.[24] Similar observations were shown among patients with peripheral arterial disease.[25]

Whilst retrospective studies provide evidence of an association, they do not prove a causal relation. Prospective studies have shown that elevations in homocysteine precede the onset of primary cardiovascular events, suggesting the role of homocysteine may be more complicated than simply being a marker of subclinical disease.

Homocysteine and diet

Polygenic factors clearly play an important role in homocysteine metabolism; levels of total plasma homocysteine may, however, also be profoundly influenced by diet. The importance of certain B vitamins as co-enzymes and substrates in homocysteine metabolism has been demonstrated by reports of an inverse relationship between homocysteine and blood levels of folate, cobalamin and pyridoxine.[26,27] A high level of plasma homocysteine is, in itself, considered to be indicative of folate/cobalamin and pyridoxine deficiencies.[28]

Meta-analysis of 12 randomised control trials estimated that a 25% reduction in elevated blood homocysteine could be achieved with mean supplementation of 0.5–5.7 mg folic acid per day (the current UK adult RNI for folate is 200 µg/day). Moreover, the addition of 0.02–1 mg/day of cobalamin resulted in a further 7% reduction.[29] This has been corroborated by a recent study which proposed that a combination of folic acid and cobalamin would be more effective at lowering homocysteine levels than folic acid alone.[30]

Importantly, vitamin supplementation appears to confer a protective effect in patients with premature peripheral occlusive disease. In particular, administration of folate, cobalamin and pyridoxine to 38 individuals with homocysteine levels > 14 µmol/L was found to cease any further advancement of carotid artery plaque area.[31] Moreover, supplementation with folic acid and pyridoxine to patients with peripheral arterial occlusive disease not only resulted in normalisation of homocysteine metabolism, but also diminished biochemical abnormalities associated with vascular dysfunction.[3] This finding implies that a reduction of homocysteine to normal levels could restore normal blood vessel function.

Supplementation may be of significant benefit to elderly patients, who tend to have higher levels of homocysteine than the general population and a greater propensity towards cardiovascular disease. Researchers in Scotland have just concluded a three-year study into the effects of folic acid supplementation on homocysteine levels in over 350 elderly patients aged 65–75 years. Only those receiving 400–600 µg/day had significantly lower homocysteine levels compared to placebo. They estimated that intakes of folic acid in elderly populations would need to be increased to 926 µg/day to avoid folate deficiency and associated cardiovascular risk.[32] High intakes of folic acid should ideally

Table 1. Overview of homocysteine-lowering clinical trials currently underway

Trial	Location	Patient population	n	Vitamin dose
Bergen Vitamin Study	Norway	Coronary heart disease	2,000	Folic acid, 5 mg *vs.* placebo
CHAOS-2 Cambridge Heart Antioxidant Study	UK	Myocardial infarction or unstable angina	4,000	Folic acid, 5 mg *vs.* placebo
NORVIT Norwegian Study of Homocysteine Lowering with B vitamins in Myocardial Infarction	Norway	Myocardial infarction	3,000	Folic acid, 5 mg *vs.* placebo
PACIFIC Prevention with A Combined Inhibitor and Folate in Coronary Heart Disease	Australia, New Zealand	High risk or previous vascular disease	10,000	Folic acid, 0.2 mg or 2 mg *vs.* placebo
SEARCH Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine	UK	Myocardial infarction	12,000	Folic acid, 2 mg + B_{12}, 1 mg *vs.* placebo
VISP Vitamin Intervention for Stroke Prevention	USA	Stroke	3,600	Folic acid, 2.5 mg + B_{12}, 0.4 mg + B_6, 25 mg *vs.* folic acid, 0.02 mg + B_{12}, 0.06 mg + B_6, 0.2 mg
VITATOPS Vitamins to Prevent Stroke	Australia	Stroke	5,000	Folic acid, 2 mg + B_{12}, 0.5 mg + B_6, 25 mg *vs.* placebo
WACS Women's Antioxidant and Cardiovascular Disease Study	USA	Vascular disease or multiple cardiovascular risk factors in women	8,000	Folic acid, 2.5 mg + B_{12}, 1 mg + B_6, 50 mg *vs.* placebo

be accompanied by supplementation with cobalamin, as folate can mask early signs of cobalamin deficiency and, as such, sub-acute nerve degeneration may progress undetected.

In all, concordance of data suggests the atherogenicity of homocysteine may be acquiescent to prevention and even remission with correct vitamin supplementation. Several large-scale clinical trials are now underway (see table 1) to assess whether homocysteine-lowering therapy will reduce cardiovascular morbidity and mortality and if the associations are, in fact, causal. Whilst these trials await completion, measurement of plasma homocysteine concentrations may identify patients with traditional risk factors who could benefit from more intensive management.

Table 2. Factors contributing to hyperhomocysteinaemia

- Increasing age
- Masculine gender
- Genetic defects in homocysteine metabolism
- Vitamin deficiencies
- Smoking
- Low physical activity levels
- Hypothyroidism
- Kidney disease
- Drugs (methotrexate, theophylline, corticosteroids, cyclosporin, phenytoin, fibrates)

Laboratory measurements of homocysteine

New and sensitive tests for measuring levels of homocysteine in the blood are becoming increasingly widespread and, in the US especially, there is growing recognition of their clinical benefit. The majority of homocysteine (70%) is bound to albumin, with the remaining 30% comprising either free homocysteine or disulphide complexes with other thiols. These forms of homocysteine are collectively referred to as 'total homocysteine' or, simply, 'homocysteine'.

For accurate assessment, it is essential that red blood cells be separated from plasma at point of collection, as homocysteine levels have been found to increase rapidly in unseparated whole blood samples at room temperature.[33] Separation cards have been purposely developed to incorporate both optimum separation and ease of use (only a few drops of finger-prick blood are necessary). Following separation, homocysteine remains stable for several days at ambient temperatures and several years at -20ºC, allowing separated samples to be forwarded to the laboratory for analysis.

Normal levels of homocysteine are purported to lie between 8 and 15 µmol/L. Levels above this are classified into moderate, intermediate and severe hyperhomocysteinaemia with concentrations between 16–30, 31–100 and > 100 µmol/L, respectively.[34] A number of factors may influence homocysteine levels (table 2) and should be given due consideration when interpreting results.

Key messages

- Clinical and epidemiological studies suggest a positive association between hyperhomocysteinaemia and cardiovascular disease
- Genetic defects in homocysteine metabolism, and dietary deficiencies of folate and B vitamins can result in increased levels of plasma homocysteine
- Reductions in plasma homocysteine can be achieved by dietary supplementation with appropriate levels of folate and B vitamins
- Measurements of plasma homocysteine may be beneficial to patients with, and at risk of, cardiovascular disease

Conclusion

Consistent reports of an association between increased homocysteine and cardiovascular morbidity and mortality underscore the need to determine homocysteine status in patients with, or at risk of, cardiovascular disease. Dietary modification strategies to reduce homocysteine levels in at-risk individuals have the potential to be of significant benefit to patients and health services alike. If homocysteine-reducing therapy is considered, a 'screen and treat' approach may prove more cost-effective than widespread supplementation programmes.

References

1. British Heart Foundation Statistics database 2002. Coronary Heart Disease Statistics. Annual Compendium: 2002 edition.
2. Voutilainen S, Morrow JD, Roberts LJ 2nd *et al*. Enhanced *in vivo* lipid peroxidation at elevated plasma total homocysteine levels. *Arterioscler Thromb Vasc Biol* 1999;**19**:1263-6.
3. Van den Berg M, Boers GH, Franken DG *et al*. Hyperhomocysteinemia and endothelial dysfunction in young patients with peripheral arterial occlusive disease. *Eur J Clin Invest* 1995;**25**:176-81.
4. Nappo F, De Rosa N, Marfella R *et al*. Impairment of endothelial function by acute hyperhomocysteinemia and reversal by antioxidant vitamins. *JAMA* 1999;**281**:2113-18.
5. Tsai JC, Perrella MA, Yoshizumi M *et al*. Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis. *Proc Natl Acad Sci USA* 1994;**91**:6369-73.
6. Lentz SR. Mechanisms of thrombosis in hyperhomocysteinemia. *Curr Opin Hematol* 1998;**5**:343-9.
7. Berg K, Malinow MR, Kierulf P, Upson B. Population variation and genetics of plasma homocyst(e)ine level. *Clin Genet* 1992;**41**:315-21.
8. Reed T, Malinow MR, Christian JC, Upson B. Estimates of heritability of plasma homocyst(e)ine levels in aging adult male twins. *Clin Genet* 1991;**39**:425-8.
9. Tonstad S, Refsum H, Sivertsen M, Christophersen B, Ose L, Ueland PM. Relation of total homocysteine and lipid levels in children to premature cardiovascular death in male relatives. *Pediatr Res* 1996;**40**:47-52.
10. Franken DG, Boers GH, Blom HJ, Cruysberg JR, Trijbels FJ, Hamel BC. Prevalence of familial mild hyperhomocysteinemia. *Atherosclerosis* 1996;**125**:71-80.
11. Tonstad S, Refsum H, Ueland PM. Association between plasma total homocysteine and parental history of cardiovascular disease in children with familial hypercholesterolemia. *Circulation* 1997;**96**:1803-08.
12. Hopkins PN, Wu LL, Wu J *et al*. Higher plasma homocyst(e)ine and increased susceptibility to adverse effects of low folate in early familial coronary artery disease. *Arterioscler Thromb Vasc Biol* 1995;**15**:1314-20.
13. Verhoef P, Kok FJ, Kruyssen DA *et al*. Plasma total homocysteine, B vitamins, and risk of coronary atherosclerosis. *Arterioscler Thromb Vasc Biol* 1997;**17**:989-95.
14. Refsum H, Ueland PM, Nygard O, Vollset SE. Homocysteine and cardiovascular disease. *Ann Rev Med* 1998;**49**:31-62.
15. Evers S, Koch HG, Grotemeyer KH, Lange B, Deufel T, Ringelstein EB. Features, symptoms, and neurophysiological findings in stroke associated with hyperhomocysteinemia. *Arch Neurol* 1997;**54**:1276-82.
16. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *JAMA* 1995;**274**:1049-57.
17. Ueland PM, Refsum H, Beresford SA, Vollset SE. The controversy over homocysteine and cardiovascular risk. *Am J Clin Nutr* 2000;**72**:324-32.
18. Graham IM, Daly LE, Refsum HM *et al*. Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. *JAMA* 1997;**277**:1775-81.
19. Stampfer MJ, Malinow MR, Willett WC *et al*. A prospective study of plasma homocysteine and risk of myocardial infarction in US physicians. *JAMA* 1992;**268**:877-81.
20. Arnesen E, Refsum H, Bonaa KH, Ueland PM, Forde OH, Nordrehaug JE. Serum total homocysteine and coronary heart disease. *Int J Epidemiol* 1995;**24**:704-09.
21. Wald NJ, Watt HC, Law MR, Weir DG, McPartlin J, Scott J. Homocysteine and ischaemic heart disease: results of a prospective study with implications regarding prevention. *Arch Intern Med* 1998;**158**:862-7.
22. Wincup PH, Refsum H, Perry IJ *et al*. Serum total homocysteine and coronary heart disease: prospective study in middle aged men. *Heart* 1999;**82**:448-54.
23. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *BMJ* 2002;**325**:1202.
24. Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med* 1997;**337**:230-6.
25. Taylor LM Jr, Moneta GL, Sexton GJ, Schuff RA, Porter JM. Prospective blinded study of the relationship between plasma homocysteine and progression of symptomatic peripheral arterial disease. *J Vasc Surg* 1999;**29**:8-21.
26. Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *JAMA* 1993;**270**:2693-8.
27. Robinson K, Arheart K, Refsum H *et al*. Low circulating folate and vitamin B6 concentrations: risk factors for stroke, peripheral vascular disease and coronary artery disease: European COMAC Group. *Circulation* 1998;**97**:437-43.
28. Ueland PM, Refsum H, Stabler SP, Malinow MR, Andersson A, Allen RH. Total homocysteine in plasma or serum: methods and clinical applications. *Clin Chem* 1993;**39**:1764-79.
29. Homocysteine Lowering Trialists' Collaboration. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. *BMJ* 1998;**316**:894-8.
30. Quinlivan EP, McPartlin J, McNulty H *et al*. Importance of both folic acid and vitamin B12 in reduction of risk of vascular disease. *Lancet* 2002;**359**:227-8.
31. Peterson JC, Spence JD. Vitamins and progression of atherosclerosis in hyperhomocyst(e)inaemia. *Lancet* 1998;**351**:263.
32. Rydlewicz A, Simpson JA, Taylor RJ, Bond CM, Golden MH. The effect of folic acid supplementation on plasma homocysteine in an elderly population. *QJM* 2002;**95**:27-35.
33. Ubbink JB, Vermaak WJ, van der Merwe A, Becker PJ. The effect of blood sample aging and food consumption on plasma total homocysteine levels. *Clin Chim Acta* 1992;**207**:119-28.
34. Malinow MR, Bostom AG, Krauss RM. Homocyst(e)ine, diet, and cardiovascular disease: a statement for healthcare professionals from the Nutrition Committee, American Heart Association. *Circulation* 1999;**99**:178-82.