REVIEW ARTICLES

Homocysteine—a novel risk factor for vascular disease

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Coronary heart disease (CHD) causes almost one-quarter of all deaths in the UK. In addition to this substantial loss of life, cardiovascular disease accounts for considerable morbidity: about 3 million people in the UK have symptomatic CHD.

Population studies in Framingham and elsewhere have identified cigarette smoking, hypertension, diabetes, age, raised total cholesterol, and low HDL cholesterol as major and independent risk factors for CHD[1]. However, these risk factors are insufficient to account for all cases of CHD, or for the differences in CHD event rates between populations[2,3]. In a proportion of patients, additional mechanisms must be operational. In this article we review the evidence implicating raised plasma homocysteine concentration as an aetiological factor in cardiovascular disease.

HOMOCYSTEINE—A NATURALLY OCCURRING AMINOACID

Homocysteine is an aminoacid derived from the metabolism of dietary methionine (Figure 1)[4]. Conversion of methionine to homocysteine is an essential, ubiquitous component of intracellular metabolism which liberates methyl groups required for the synthesis of DNA, steroid hormones and some proteins. In plasma, most of the homocysteine is protein bound, with the remaining free homocysteine existing in reduced and oxidized forms[5]. In the laboratory total plasma homocysteine concentrations are usually measured by high-performance liquid chromatography[6], but a recently introduced immunoassay offers a rapid, accurate assessment more suitable for routine clinical use[7].

Normal levels of total plasma homocysteine vary between 5 and 15 μmol/L. Mutations in the enzymes involved in homocysteine metabolism, and deficiencies of vitamins B_6, B_{12} and folic acid (co-factors for these enzymes), are associated with hyperhomocysteinaemia[4,8]. The 677C→T mutation in the enzyme 5,10-methylene-tetrahydrofolate reductase (MTHFR), renders the enzyme thermolabile and functionally impaired[9]. Homozygosity for 677T MTHFR is found in approximately 15% of European whites, and is associated with a 2–3 μmol/L increase in homocysteine concentrations[10]. Dietary factors also have a major influence on levels of homocysteine. Oral methionine, derived from dietary protein, induces short-term increments in homocysteine concentrations[11], and in elderly populations low levels of B vitamins may be the primary determinant of raised plasma homocysteine[12]. Hyperhomocysteinaemia is also more prevalent amongst patients with renal impairment, recipients of renal and heart transplants and patients treated with drugs such as methotrexate which interfere with folate metabolism[8,13,14].

HOMOCYSTINURIA AND VASCULAR DISEASE

The possible clinical importance of homocysteine was first recognized in the 1960s, after description of the rare metabolic disorder homocystinuria. Affected children, who have severe hyperhomocysteinaemia (plasma concentrations >100 μmol/L), develop widespread premature atherosclerosis[15]. If untreated, they usually die of vascular complications such as pulmonary embolism, myocardial infarction, cerebrovascular accidents, and peripheral arterial thromboses[16,17]. The vascular pathology is similar to that of conventional adult atherosclerosis, although intimal

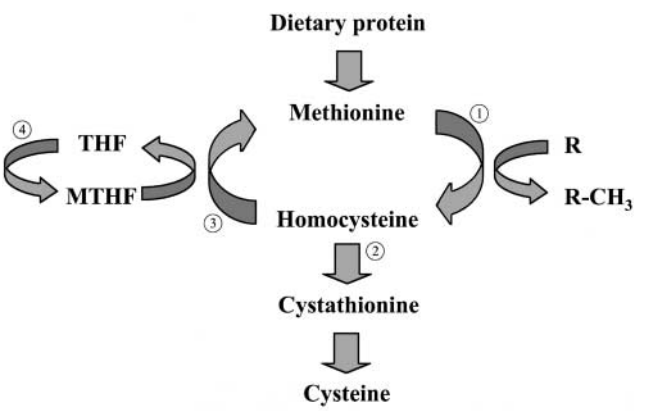

Figure 1 **Outline of metabolic pathway for homocysteine.** (1) Trans-methylation: conversion of methionine to homocysteine, thereby transferring methyl group to other species (R). (2) Trans-sulphuration: irreversible conversion of homocysteine to cysteine: via rate limiting enzyme cystathionine-β-synthase, with vitamin B_6 as essential co-factor. (3) Re-methylation: regeneration of methionine from homocysteine: catabolized by methionine synthase, with 5,10-methylene-tetrahydrofolate (MTHF, a form of folic acid) and vitamin B_{12} as essential co-factors. (4) Regeneration of MTHF from tetrahydrofolate (THF), catabolized by enzyme 5,10-methylene-tetrahydrofolate reductase

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thickening and fibrous plaque are more common and there is a strong propensity for intravascular thrombosis[16]. Restriction of dietary methionine intake together with B vitamin supplementation (pyridoxine, folate and B_{12}) is effective in lowering homocysteine levels in some children with homocystinuria[17]. Observations that these treatment regimens substantially reduce cardiovascular risk in homocystinuric patients[17] support the view that raised homocysteine concentrations have a causal role in the vascular pathology of homocystinuria.

HOMOCYSTEINE AND RISK OF VASCULAR DISEASE IN ADULTS

Recognition that children with homocystinuria develop aggressive premature vascular disease led to the hypothesis that mild-to-moderate increases in plasma homocysteine might contribute to the development and progression of atherosclerosis in adults. This hypothesis has now been evaluated in over thirty cross-sectional and prospective studies involving more than 10 000 individuals[18,19]. The results have been remarkably consistent. Raised plasma homocysteine is an independent risk factor for peripheral vascular, cerebrovascular, and coronary heart disease. The increased risk of CHD associated with homocysteine is additive to that of conventional vascular risk factors[20], and is present even within the normal range for homocysteine[21]. In North American and European populations, a 5 μmol/L increase in homocysteine concentrations has been judged equivalent to an 0.6 mmol/L rise in total cholesterol, with raised homocysteine contributing to 10% of population CHD risk[18].

DIFFERENCES IN HOMOCYSTEINE BETWEEN PATIENT GROUPS

Observations that plasma homocysteine is raised in patients with renal failure and in transplant recipients suggest that homocysteine may contribute to the widespread atherosclerosis and the high incidence of cardiovascular events seen in these patients[13,14]. Plasma homocysteine concentrations vary not only between patient groups but also between countries, and in Europe are closely related to national age-standardized cardiovascular disease mortality rates[22]. Although few studies have been undertaken in non-white populations, recent data show that Indian Asians have higher plasma homocysteine concentrations than European whites—a difference that might contribute to their higher CHD mortality rates[23,24]. Conversely, South African blacks have more effective homocysteine metabolism and lower homocysteine levels than South African whites, which may explain their relative resistance to CHD despite a high prevalence of obesity, hypertension and smoking[25].

MECHANISMS LINKING HOMOCYSTEINE TO VASCULAR DISEASE

There is continuing uncertainty whether homocysteine has a causal role in the development of atherosclerosis or is simply a marker for increased vascular risk[26]. Evidence to support a direct role for homocysteine in the pathogenesis of vascular disease has emerged from studies showing a dynamic and inverse relationship between plasma homocysteine and vascular endothelial function. Acute hyperhomocysteinaemia is associated with rapid-onset vascular

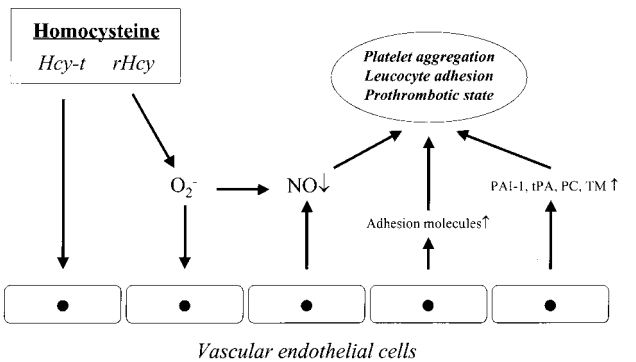

Figure 2 **Putative mechanisms linking homocysteine to vascular disease**. Exposure of vascular endothelial cells to raised homocysteine concentration leads to reduced nitric oxide (Refs 28, 32), increased levels of adhesion molecules (Ref. 48) and expression of procoagulant species including plasminogen activator inhibitor-1 (PAI-1), tissue plasminogen activator (tPA), protein C (PC) and thrombomodulin (TM) (Ref. 49). These actions may promote platelet aggregation, leucocyte adhesion and thrombosis. The effects of homocysteine may be mediated by the free reduced form of homocysteine (rHcy), which promotes generation of superoxide (O_2^-), hydrogen peroxide and other oxygen-derived free radicals (Refs 32, 35). Recent studies have suggested an additional effect of homocysteine thiolactone (Hcy-t) on endothelial cellular function, mediated by protein homocysteinylation (Refs 39, 40).

endothelial dysfunction, an early manifestation of atherosclerosis[27–31]. These observations are consistent with reports of dose and time dependent effects of homocysteine on endothelial cellular function *in vitro*[32–34]. Endothelial dysfunction can also be induced by the increments in plasma homocysteine that follow low-dose oral methionine, or dietary consumption of animal protein[29]. These findings suggest that even diet-related increments in plasma homocysteine contribute to the development and progression of atherosclerosis.

The possible mechanisms linking homocysteine to endothelial dysfunction are summarized in Figure 2. Homocysteine may exert its effects by promoting oxidative damage in endothelial cells, with the generation of superoxide anion radicals (O_2-) and hydrogen peroxide (H_2O_2)[32,35]. Generation of free-radical superoxide anions may promote oxidation of low-density lipoprotein (LDL) and deactivation of nitric oxide[36]. Deactivation of nitric oxide, the major endothelium-derived vasodilator, may lead to vasoconstriction, platelet aggregation and monocyte adhesion, all of which promote atherosclerosis[36]. The

presence of oxidant stress during hyperhomocysteinaemia is confirmed by measurement of lipid peroxides[37,38] and also by the effect of the antioxidant vitamin C, which reverses homocysteine-induced endothelial dysfunction[28,31]. More recent *in-vitro* studies have suggested that homocysteine reacts with amine groups on proteins, possibly producing abnormalities of enzyme or receptor function[39,40]. The *in-vivo* significance of this protein homocysteinylation remains to be determined.

EFFECTS OF HOMOCYSTEINE LOWERING

Folic acid and vitamin B_{12} are essential cofactors for the remethylation of homocysteine to methionine, and dietary supplementation with these B vitamins provides an effective means to lower concentrations of plasma homocysteine[41]. In adults, dietary folic acid at doses of 0.5–5 mg/day lowers homocysteine concentrations by about 25%. Additional oral vitamin B_{12} results in a further 7% reduction in plasma homocysteine, when given at a dose of 0.5 mg/day. Epidemiological data suggest that a decrease in homocysteine concentrations of this magnitude might reduce risk of cardiovascular events in patients with CHD by as much as 15–30%[42]. However, at present little is known about the possible beneficial vascular effects of lowering homocysteine in man.

Recent studies have examined the effects of homocysteine lowering on surrogate markers of atherosclerosis. In healthy volunteers and in patients with CHD, B vitamin supplementation is associated with an improvement in endothelium-dependent dilatation and in serum markers of endothelial injury[7,43–46]. Furthermore, amongst healthy siblings of patients with premature atherosclerosis, homocysteine lowering reduces the occurrence of abnormal exercise tests to an extent consistent with a decreased risk of future atherosclerotic coronary events[47]. More conclusive evidence on the causal role of homocysteine in vascular disease will emerge from large-scale randomized placebo-controlled intervention trials now underway. The aim is to discover whether homocysteine lowering will reduce the incidence of major cardiovascular events in patients with CHD[42]. The results of these intervention trials, expected within 5 years, are required before we could advocate routine screening of patients with vascular disease for homocysteine levels, or widespread use of vitamin therapy for the prevention of cardiovascular disease.

CONCLUSIONS

Epidemiological studies provide convincing and consistent evidence that raised homocysteine is a risk factor for vascular disease, including CHD, in adults. Physiological studies show that a high plasma homocysteine induces vascular endothelial dysfunction, so the relationship between homocysteine and vascular disease may well be causal. However, at present there are no data to show that lowering homocysteine will reduce major cardiovascular endpoints.

REFERENCES

1 Grundy SM, Pasternak R, Greenland P, Smith S Jr, Fuster V. AHA/ACC scientific statement. Assessment of cardiovascular risk by use of multiple-risk-factor assessment equations: a statement for healthcare professionals from the American Heart Association and the American College of Cardiology. *J Am Coll Cardiol* 1999;**34**:1348–59

2 van den Hoogen PC, Feskens EJ, Nagelkerke NJ, Menotti A, Nissinen A, Kromhout D. The relation between blood pressure and mortality due to coronary heart disease among men in different parts of the world. Seven Countries Study Research Group. *N Engl J Med* 2000;**342**:1–8

3 Anand SS, Yusuf S, Vuksan V, *et al.* Differences in risk factors, atherosclerosis, and cardiovascular disease between ethnic groups in Canada: the Study of Health Assessment and Risk in Ethnic groups (SHARE). *Lancet* 2000;**356**:279–84

4 Scott JM, Weir DG. Folic acid, homocysteine and one-carbon metabolism: a review of the essential biochemistry. *J Cardiovasc Risk* 1998;**5**:223–7

5 Ueland PM. Homocysteine species as components of plasma redox thiol status. *Clin Chem* 1995;**41**:340–2

6 Fiskerstrand T, Refsum H, Kvalheim G, Ueland PM. Homocysteine and other thiols in plasma and urine: automated determination and sample stability. *Clin Chem* 1993;**39**:263–71

7 Pfeiffer CM, Twite D, Shih J, Holets M Sr, Gunter EW. Method comparison for total plasma homocysteine between the Abott IMx analyzer and an HPLC assay with internal standardization. *Clin Chem* 1999;**45**:152–3

8 Mayer EL, Jacobsen DW, Robinson K. Homocysteine and coronary atherosclerosis. *J Am Coll Cardiol* 1996;**27**:517–27

9 Frosst P, Blom HJ, Milos R, *et al.* A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995;**10**:111–13

10 Brattstrom L, Wilcken DE, Ohrvik J, Brudin L. Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis. *Circulation* 1998;**98**:2520–6

11 Guttormsen AB, Schneede J, Fiskerstrand T, Ueland PM, Refsum HM. Plasma concentrations of homocysteine and other aminothiol compounds are related to food intake in healthy human subjects. *J Nutr* 1994;**124**:1934–41

12 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

13 Gupta A, Moustapha A, Jacobsen DW, *et al.* High homocysteine, low folate, and low vitamin B6 concentrations: prevalent risk factors for vascular disease in heart transplant recipients. *Transplantation* 1998;**65**:544–50

14 Bostom AG, Gohh RY, Tsai MY, *et al.* Excess prevalence of fasting and postmethionine-loading hyperhomocysteinemia in stable renal transplant recipients. *Arterioscler Thromb Vasc Biol* 1997;**17**:1894–900

15 McCully KS. Vascular pathology of homocyst(e)inemia: implications for the development of arteriosclerosis. *Am J Pathol* 1969;**56**:111–28

16 Mudd SH, Skovby F, Levy HL, Pettigrew KR, Wilcken B, Pyeritz R. The natural history of homocystinuria due to cystathionine b-synthase deficiency. *Am J Hum Genet* 1985;**37**:1–31

17 Wilcken DE, Wilcken B. The natural history of vascular disease in homocystinuria and the effects of treatment. *J Inherit Metab Dis* 1997;**20**:295–300

18 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

19 Danesh J, Lewington S. Plasma homocysteine and coronary heart disease: systematic review of published epidemiological studies. *J Cardiovasc Risk* 1998;**5**:229–32

20 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

21 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

22 Alfthan G, Aro A, Gey KF. Plasma homocysteine and cardiovascular disease mortality. *Lancet* 1997;**349**:397

23 Chambers JC, Obeid OA, Refsum H, *et al.* Plasma homocysteine concentrations and risk of coronary heart disease in UK Indian Asian and European men. *Lancet* 2000;**355**:523–7

24 Chambers JC, Ireland H, Thompson E, *et al.* The methylenetetrahydrofolate reductase 677 C→T mutation, and coronary heart disease risk in UK Indian Asians. *Arterioscler Thromb Vasc Biol* (in press)

25 Ubbink JB, Delport R, Vermaak WJ. Plasma homocysteine concentrations in a population with a low coronary heart disease prevalence. *J Nutr* 1996;**126**:1254S–7S

26 Dudman NP. An alternative view of homocysteine. *Lancet* 1999;**354**: 2072–4

27 Chambers JC, McGregor A, Jean Marie J, Kooner JS. Acute hyperhomocysteinaemia and endothelial dysfunction. *Lancet* 1997; **351**:36–7

28 Chambers JC, McGregor A, Jean Marie J, Obeid OA, Kooner JS. Demonstration of rapid onset vascular endothelial dysfunction after hyperhomocysteinemia: an effect reversible with vitamin C therapy. *Circulation* 1999;**99**:1156–60

29 Chambers JC, Obeid OA, Kooner JS. Physiological increments in plasma homocysteine induce vascular endothelial dysfunction in normal human subjects. *Arterioscler Thromb Vasc Biol* 1999;**19**:2922–7

30 Bellamy MF, McDowell IF, Ramsey MW, *et al.* Hyperhomocysteinemia after an oral methionine load acutely impairs endothelial function in healthy adults. *Circulation* 1998;**98**:1848–52

31 Kanani PM, Sinkey CA, Browning RL, Allaman M, Knapp HR, Haynes WG. Role of oxidant stress in endothelial dysfunction produced by experimental hyperhomocysteinemia in humans. *Circulation* 1999; **100**:1161–8

32 Stamler JS, Osborne JA, Jaraki O, *et al.* Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. *J Clin Invest* 1993;**91**:308–18

33 Blundell G, Jones BG, Rose FA, Tudball N. Homocysteine mediated endothelial cell toxicity and its amelioration. *Atherosclerosis* 1996;**122**: 163–72

34 Dudman NP, Temple SE, Guo XW, Fu W, Perry MA. Homocysteine enhances neutrophil–endothelial interactions in both cultured human cells and rats in vivo. *Circ Res* 1999;**84**:409–16

35 Loscalzo J. The oxidant stress of hyperhomocyst(e)inemia. *J Clin Invest* 1996;**98**:5–7

36 Kojda G, Harrison D. Interactions between NO and reactive oxygen species: pathophysiological importance in atherosclerosis, hypertension, diabetes and heart failure. *Cardiovasc Res* 1999;**43**:562–71

37 Ventura P, Panini R, Verlato C, Scarpetta G, Salvioli G. Peroxidation indices and total antioxidant capacity in plasma during hyperhomocysteinemia induced by methionine oral loading. *Metabolism* 2000;**49**:225–8

38 Voutilainen S, Morrow JD, Roberts LJ, *et al.* Enhanced in vivo lipid peroxidation at elevated plasma total homocysteine levels. *Arterioscler Thromb Vasc Biol* 1999;**19**:1263–6

39 Jakubowski H, Zhang L, Bardeguez A, Aviv A. Homocysteine thiolactone and protein homocysteinylation in human endothelial cells: implications for atherosclerosis. *Circ Res* 2000;**87**:45–51

40 Jakubowski H. Protein homocysteinylation: possible mechanism underlying pathological consequences of elevated homocysteine levels. *FASEB J* 1999;**13**:2277–83

41 Homocysteine Lowering Trialists' Collaboration. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. *BMJ* 1998;**316**:894–8

42 Clarke R, Collins R. Can dietary supplements with folic acid or vitamin B6 reduce cardiovascular risk? Design of clinical trials to test the homocysteine hypothesis of vascular disease. *J Cardiovasc Risk* 1998; **5**:249–55

43 Woo KS, Chook P, Lolin YI, Sanderson JE, Metreweli C, Celermajer DS. Folic acid improves arterial endothelial function in adults with hyperhomocystinemia. *J Am Coll Cardiol* 1999;**34**:2002–6

44 Bellamy MF, McDowell IF, Ramsey MW, Brownlee M, Newcombe RG, Lewis MJ. Oral folate enhances endothelial function in hyperhomocysteinaemic subjects. *Eur J Clin Invest* 1999;**29**:659–62

45 van den BM, Boers GH, Franken DG, *et al.* Hyperhomocysteinaemia and endothelial dysfunction in young patients with peripheral arterial occlusive disease. *Eur J Clin Invest* 1995;**25**:176–81

46 Chambers JC, Ueland PM, Obeid OA, Wrigley J, Refsum H, Kooner JS. Improved vascular endothelial function after oral B vitamins; an effect mediated through reduced concentrations of free plasma homocysteine. *Circulation* (in press)

47 Vermeulen EG, Stehouwer CD, Twisk JW, *et al.* Effect of homocysteine-lowering treatment with folic acid plus vitamin B6 on progression of subclinical atherosclerosis: a randomised, placebo-controlled trial. *Lancet* 2000;**355**:517–22

48 Nappo F, De Rosa N, Marfella R, *et al.* Impairment of endothelial functions by acute hyperhomocysteinemia and reversal by antioxidant vitamins. *JAMA* 1999;**281**:2113–18

49 Lentz SR, Sobey CG, Piegors DJ, *et al.* Vascular dysfunction in monkeys with diet-induced hyperhomocyst(e)inemia. *J Clin Invest* 1996;**98**:24–9