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REVIEW ARTICLE

Homocysteine in Myointimal Hyperplasia

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Introduction: *homocysteine, a sulphur-containing non-essential amino acid, appears to play a role in the pathophysiology of atherosclerosis. However, its role in myointimal hyperplasia, the cause of almost 30% of failures of interventional therapeutic procedures, is much less clear.*
Methods: *a review of the published scientific data concerning the role of homocysteine in myointimal hyperplasia was performed using MEDLINE and other on-line databases. Evidence was sought from cell culture experiments, animal models and clinical studies.*
Results: *several clinical studies have recently been published linking plasma homocysteine levels to restenosis in coronary and peripheral arterial disease. However, several contradictory studies also exist making the role of homocysteine unclear. There are currently no published randomised trials. Cell culture and animal model experiments have elucidated several potential mechanisms by which may stimulate myointimal hyperplasia. Possible mechanisms include endothelial cell activation with the enhanced release of inflammatory cytokines and growth factors and a direct effect on vascular smooth muscle cell migration and proliferation.*
Conclusion: *further studies are required before the true role of homocysteine in the pathogenesis of myointimal hyperplasia can be clearly evaluated. If evidence does confirm a role, the ease with which homocysteine levels can be normalised makes it an attractive alternative therapeutic target for intervention.*

Key Words: Homocysteine; Myointimal hyperplasia; Restenosis; Review.

Introduction

Balloon angioplasty and surgical bypass techniques have revolutionised the management of patients with coronary and peripheral vascular disease. However, approximately a third of these patients will develop restenosis[1,2] mainly due to the development of myointimal hyperplasia (MIH). Recently the non-essential amino acid homocysteine (HCy) has been implicated as a possible risk factor in premature graft and angioplasty restenosis.[3–5] This has generated particular interest as plasma levels of HCy can be normalised simply and cheaply by the intake of folic acid and the vitamins B_6 and B_{12}.[6,7] However, the precise role of HCy in the development of MIH remains unclear and will be the subject of this review.

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Myointimal Hyperplasia

MIH can be considered an exaggerated healing response to injury. Activated endothelial cells initiate the inflammatory response via platelet activation, or via the direct release of cytokines and growth hormones. This leads to a cascade of effects which result in: migration of vascular smooth muscle cells (VSMC) from the media into the intima,[8,9] VSMC proliferation and phenotypic change to a more secretory fibroblastic cell type[10] and continued production of extracellular matrix (ECM), in particular collagen, in excess of degradation;[11] which eventually leads to stenosis of the increasingly non-compliant vessel wall. Continued trauma from the presence of sutures; shear stresses;[12,13] or low flow of blood in areas of turbulence perhaps leading to a local concentration of harmful irritants in the blood, may then act to maintain the endothelium in an activated state. This leads to continuing platelet and macrophage activation even after the endothelial monolayer has regenerated.

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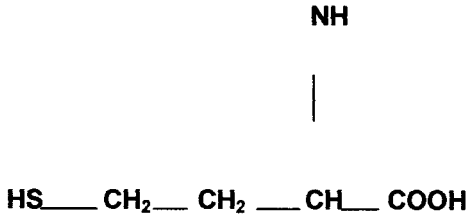

Fig. 1. Structure of homocysteine.

Homocysteine

HCy is a non-essential, non-protein forming, sulphur-containing amino acid, produced from the metabolism of dietary methionine (Fig. 1). The metabolic pathways involving HCy are illustrated in Figure 2. It is found in the serum as protein-bound HCy (70%), HCy-HCy and HCy-Cysteine mixed disulphides (30%) or as free HCy thiolactone (1%). Elevations of plasma total HCy have a multi-factorial basis, resulting from a combination of increased dietary intake of methionine, inheritable genetic defects and vitamin deficiencies (Fig. 2).[14] Severe homocysteinaemia (>100 μmol/l) results from rare homozygous genetic defects such as deficiency of cystathionine β synthase (CβS), methylenetetrahydrofolate reductase (MTHFR) or of enzymes involved in methyl-B_{12} synthesis and homocysteine methylation. Mild (15–30 μmol/l) to moderate (30–100 μmol/l) homocysteinaemia is due to vitamin deficiencies, heterozygous CβS defect, or MTHFR thermolability. These later genetic defects have been found to affect a third of some populations, whilst vitamin deficiencies play a large role in the elevated levels of HCy found in the elderly population.[15–17]

Free HCy and HCy-th are highly reactive molecules, forming non-covalent and covalent bonds with a variety of molecules, in particular cysteine-rich or other thiol moieties.[18,19] This can lead to loss of electrical charge, conformational changes or even precipitation of the protein, resulting in loss or degradation of the biological function of multiple enzymes, receptors, growth factors and structural proteins.[20,21] HCy is also known to activate and inactivate specific mRNA transcription and stimulate DNA synthesis directly perhaps via a specific receptor, which initiates calcium fluxes in an as yet unclear mechanism.[22,23]

The role of the endothelial cell (EC) is central to the aetiology of MIH.[24] Endothelial cells have a lower basal intracellular HCy concentration and appear to be more influenced by extracellular concentrations of the amino-acid, suggesting an increased sensitivity/decreased ability to metabolise it than other cells.[25] At physiological concentrations HCy is able to inhibit EC cell proliferation at the same time as stimulating VSMC proliferation.[26,27] Although, studies have also shown increased EC turnover and aging in response to elevated plasma HCy.[24,28] HCy activates and damages endothelial cells by the generation of reactive oxygen species[29] combined with the removal of EC protective antioxidant mechanisms such as nitric oxide (NO)[30] and glutathione[31] thus potentiating the injurious effect and increasing activation. In VSMC (see Figure 3) HCy stimulates proliferation through a variety of means probably via a specific NMDA-like receptor(s).[32] These initiate several cellular cascades:

- The induction of cyclin D1 and A in a dose-dependant manner stimulates the re-entry of the normally quiescent cells into the cell cycle[26] via cyclin-dependant kinase,[33]
- Activation of phospholipases such as C and D, the synthesis of diacylglycerol (DAG) and inositol triphosphate (IP3) leads to an increase in intracellular calcium ions and activation of protein kinase C (PKC). Both of these intermediaries are capable of activating mitogen activating protein (MAP) kinase which leads to activation of DNA transcription factors and cellular proliferation,[22,32]
- Redox-specific and dose-dependant PKC activation leads to transcription of c-myb and c-fos mRNA which code for proteins that combine with others to form AP-1 which in turn initiates transcription of genes involved in cellular proliferation,[34]
- Potentiation of the mitogenic effect of angiotensin II[35] and possibly other growth factors.

HCy directly inhibits the production and extracellular release of specific proteins. This stimulates a decrease in the release of heparin-like compounds, which are potent inhibitors of VSMC proliferation,[36] as well as producing a highly procoagulant milieu through the modulation of elements of the coagulation and fibrinolytic pathways.[37–40] The coagulation/fibrinolytic pathways are further modulated through chemical interactions with other cysteine moieties present in the protein structure of many proteins involved in these pathways. HCy is in addition able to modulate the activity of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) leading to elastin degradation and accumulation of collagen within the intima and media in a dose-dependant manner.[41,42]

Recent studies have shown that, *in vitro* and *in vivo*, HCy has a direct effect on vasodilatation selectively inhibiting endothelium-dependant vasodilatation, predominantly through inhibition of NO.[43,44]

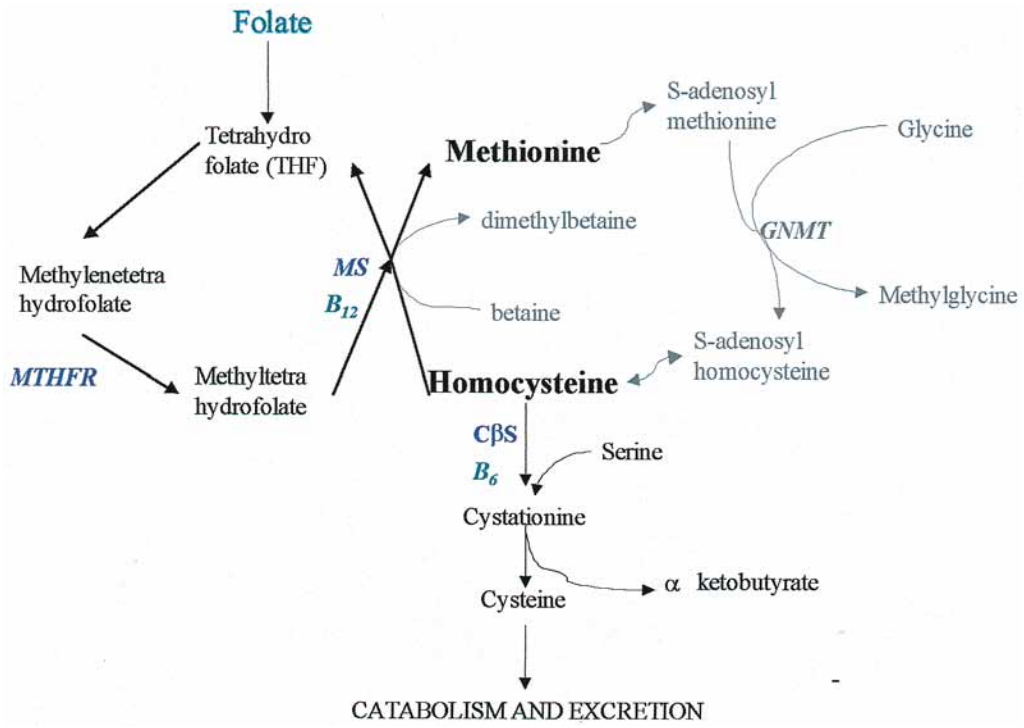

Fig. 2. Homocysteine metabolism in man. Key: MS = methionine synthase, MTHFR = methylene tetrahydrofolate reductase, CβS = cystathionine β synthase, GNMT = glycine N-methyltransferase.

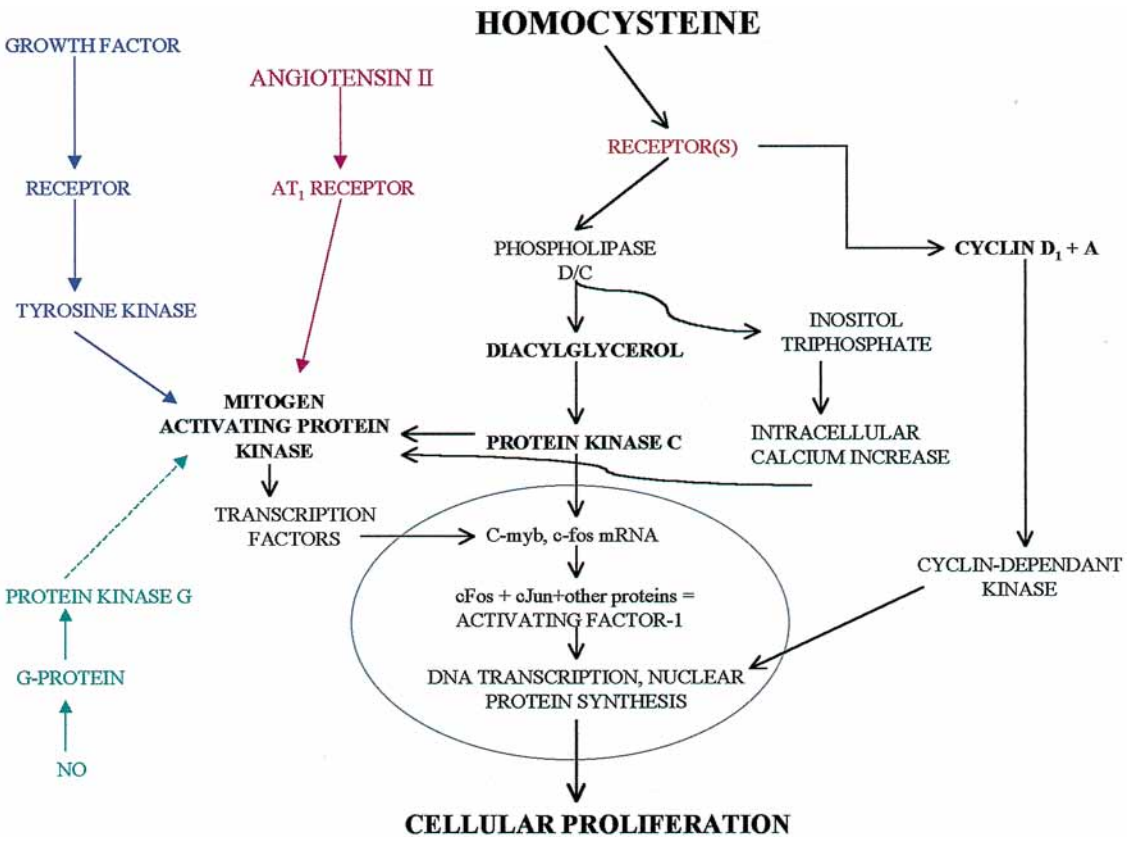

Fig. 3. Mechanisms by which cellular proliferation may be induced by homocysteine in vascular smooth cells.

Homocysteine and Clinical Restenosis

An association between elevated blood levels of homocysteine (HCy) and cardiovascular disease was originally postulated in 1969.[45] Thirty percent of patients presenting to vascular clinics have elevated plasma HCy, compared to 2–5% in the normal population.[46] There has been a deluge of studies assessing HCy as a risk factor for cardiovascular disease in the last 10 years. Boushey *et al.*[47] published a meta-analysis of 27 studies to 1995, looking at almost 4000 patients. They found that HCy was an independent, graded risk factor for atherosclerotic disease in coronary, cerebral and peripheral vessels. They calculated the odds ratio of a 5 μmol/l increment of plasma HCy level for coronary artery disease (CAD) of 1.6, cerebrovascular disease (CVA) of 1.5 and for peripheral vascular disease (PVD) of 6.8 although this value is likely to be inaccurate due to the small number of trials involving PVD available for analysis. These results have been supported by a further 40 subsequent studies[48] and will not be considered further here. The evidence for the role of HCy in premature restenosis post therapeutic revascularisation by vein graft or angioplasty is, in comparison, much less clear. However, studies do exist in both coronary and peripheral vascular disease and will be reviewed. There are currently no published studies of the association between plasma HCy and restenosis in cerebrovascular disease.

Homocysteine and coronary restenosis

Iwama[4] in a study of 40 patients who had undergone saphenous vein coronary artery bypass grafting (CABG) 1–13 years (mean 6.1 ± 3.1 years) previously, found that patients with MIH ($n = 23$) in any graft (defined as angiographic stenosis $\geq 50\%$) had significantly elevated plasma HCy levels (median 15.1 vs 10.6 μmol/l) compared to those with no evidence of restenosis. Using multiple regression analysis they were able to show that elevated HCy appeared to be an independent risk factor for vein graft stenosis. However, this retrospective study may be misleading as it supposes that levels of HCy remain relatively unchanged over several years, which is as yet unestablished. Eritsland,[49] in a prospective cohort study of 565 patients undergoing CABG in Norway found no such association between the preoperative HCy levels and the frequency of graft occlusions (determined by angiography) at 1-year. Unfortunately they did not exclude stenoses occurring within the first 6 weeks, which are usually due to thrombosis and unlikely to be due to MIH. Benoit[50] in a prospective trial of 222 patients undergoing coronary angioplasty in France who were followed up clinically for 6 months, found no significant differences in homocysteine levels between patients with multiple restenosis ($n = 79$; diagnosed by coronary angiography in 55 cases, by myocardial scintigraphy in 23 cases and strongly suspected clinically in one patient) or those requiring revascularisation, and those without restenosis and not requiring revascularisation. Morita *et al.*[51] consecutively enrolled 112 male patients who had undergone a successful elective coronary artery angioplasty and measured plasma HCy at follow-up angiography (3–6 months). They found that HCy levels were significantly higher (15.0 vs 13.0 μmol/l) in patients with restenosis (final stenosis >50% more than 3 months post angioplasty). Finally, HCy was found to be significantly higher (23.6 ± 7.8 versus 16.9 ± 7.1 μmol/l) in a case–control study of heart transplant patients who developed graft vasculopathy, defined as coronary stenosis >25% or aneurysms, as opposed to those who did not.[52] Blood was sampled a mean of 52 weeks post transplant.

Homocysteine and restenosis in peripheral vascular disease

Irvine[3] in a retrospective case-control study of 19 patients (median age 70 years at intervention) who had undergone infrainguinal vein bypass grafting and had developed stenosis between 6 weeks and 1 year post-operatively (defined as angiographically determined lumen reduction of >50% or with a persistent duplex abnormality (minimum of 2 scans) with a doubling of velocities) matched against similar patients ($n = 19$) who had not, found plasma HCy to be significantly elevated in those who later developed stenoses (median 17.8 μmol/l vs 13.8 μmol/l). Miresкandari[5] performed a similar study prospectively in 62 limbs (median age 72 years) undergoing femorodistal vein bypass. They confirmed that elevated plasma HCy was a risk factor for restenosis at 12 months (median 14.4 μmol/l, range 9.6–32 vs 9.5 μmol/l). However, they do not state whether or not they excluded stenoses occurring within the first 6 weeks. Interestingly, Beattie *et al.*[53] found hyperhomocysteinaemia (>12.2 μmol/l in women and >15.5 μmol/l in men) in 57% of 57 patients undergoing infrainguinal vein bypass surgery. Nonetheless they found that plasma HCy levels were not associated with restenosis/outcome directly in these patients, but that it was significantly associated with the development of pre-existing MIH in the vein and this,

in turn, was associated with an increased risk of graft failure. Tsakiris *et al.*[54] also found no correlation between HCy levels and rates of restenosis in 71 patients (age 68 ± 13 years) undergoing percutaneous angioplasty of predominantly femoropopliteal segments of artery in patients with PVD (52% of whom had elevated HCy levels). Currie[55] reviewed 66 patients selected retrospectively and correlated HCy levels with failure of vascular intervention, which they defined as, return of symptoms with a fall in ankle-brachial pressure index, requirement for further revascularisation procedure in the same limb or amputation. Using multiple logistic regression they found hyperhomocysteinaemia to be an independent risk factor for the failure of all forms of peripheral vascular intervention, including angioplasty, endarterectomy and bypass grafting, along with young age at intervention and diabetes.

Unfortunately, these studies do not as yet give us a clear picture of the strength of the link between elevated HCy levels and MIH. Differences in the timing of blood taken for HCy levels, the length of time to follow-up, differences in outcomes assessed, possible publication biases and the relatively small number of studies overall mean no definitive conclusions can be reached.

Restenosis in animal models of hyperhomocysteinaemia

A number of mammals have been used as models for the effects of elevated HCy in man. Baboons continuously infused with HCy developed sustained aortic EC loss and regeneration, platelet consumption and intimal lesion formation typical of arteriosclerotic or preatherosclerotic intimal lesions composed of proliferating VSMC surrounded by collagen, elastin fibres, glycosaminoglycans and sometimes lipid.[24] Similar histological changes have been reproduced in rats,[56] minipigs[57] and rabbits[58] fed methionine-rich diets. Matthias further demonstrated the potentiation of HCy-induced damage by other atherogenic substances such as cholesterol, angiotensin II, cholestane, cholic acid and methylthiouracil in spontaneously hypertensive rats. Young[59] found an increase in malondialdehyde, a major secondary oxidation product of polyunsaturated lipids, in nitrous oxide-induced hyperhomocysteinaemic pigs, whilst increased markers of lipid peroxidation were also noted in hyperhomocystaemic rabbits.[58] However, plasma HCy levels were far in excess of that usually found in hyperhomocysteinaemic patients and in fact, *in vivo* lipid peroxidation has not been demonstrated in man.[60]

Table 1. Therapeutic options in hyperhomocysteinaemia.

- Lifestyle changes
- Folic acid
- Pyroxidine (B_6)
- Vitamin B_{12}
- Betaine
- Vitamin C/Vitamin E

Monkeys fed an "atherogenic diet" developed impaired EC-dependent vasodilatation in resistance vessels of the lower limb[44] and defects in the protein C anticoagulant pathway.[61] These atherosclerotic hyperhomocysteinaemic monkeys when fed vitamin B supplemented atherosclerotic diet for 6 months demonstrated a significant fall in plasma HCy levels (12.8 ± 2.8 to 3.5 ± 0.3 μmol/l), though this did not normalise vascular function or prevent progression of structural lesions. This may be due to the fact that the elevation in HCy caused by the diet was small, however, it is more likely that this lack of effect was due to the persistent hypercholesterolaemia induced by the atherosclerotic diet, which was not affected by the vitamin supplementation. Zulli *et al.*[62] demonstrated a synergistic effect of the combination of diet-induced hyperhomocysteinaemia and hypercholesterolaemia in rats as measured by aortic wall thickness and disintegration of the elastic lamina when compared against the effects of feeding with each agent alone.

Hyperhomocysteinaemia also induced procoagulant effects,[63,64] increased prostaglandin synthesis,[65] increased diapedesis through mesenteric post capillary venules[66] and increased cyclin-dependant kinase in rats.[33]

Although these studies demonstrate that HCy can initiate or maintain endothelial and vessel wall injury, there is at present only one study looking specifically at MIH in animals: Southern *et al.*[67] studied the effects of HCy on restenosis in carotid endarterectomised rats. They found a 4-fold increase in MIH in the diet-induced hyperhomocysteinaemic group (36.32 ± 15.28 μmol/l) when calculated as percent lumen stenosis and a 8-fold increase in MIH area when compared to normals. Using a linear regression model to compare HCy levels and percent stenosis they were able to demonstrate a linear relationship between the two.

Therapeutic Options in Hyperhomocysteinaemia (Table 1)

To date, therapeutic attempts at preventing MIH have been unsatisfactory. Homocysteine provides a particularly attractive therapeutic target as blood levels

can be easily normalised with the administration of cheap, patient-acceptable, oral vitamin treatment. The implementation of a "cardiovascular lifestyle" is particularly apt, with decreased red meat intake, increased fruit and fibre, low coffee consumption, smoking cessation and regular exercise which have all been shown to decrease plasma HCy levels.[68] Both Brattstrom *et al.*[6] and Clarke *et al.*[69] in reviews of randomised controlled trials of the effects of folic acid $\pm$ pyridoxine and B_{12} administration on lowering plasma HCy levels, found that combined vitamin treatment could significantly reduce plasma HCy levels. Among these vitamins folic acid had the most potent effect: the higher the pretreatment plasma HCy and the lower the pretreatment plasma folic acid level the greater the effect. *In vitro* and *in vivo* studies have demonstrated empirical reduction in HCy-induced EC damage, VSMC proliferation and inhibition of vasodilatation when any of these vitamins were added.[70–72] There are currently no defined guidelines as to the recommended doses of these vitamins, or the level of HCy at which treatment should be initiated.[6,68] Wilcken *et al.*[73] have also demonstrated that betaine therapy (6g per day) can reduce HCy levels to normal in patients with homocystinuria and were further able to show that normalising HCy levels led to a significant decrease in vascular events in these patients.[74] Vitamin C and vitamin E, natural antioxidants, appear to ameliorate the oxidative damage caused by hyperhomocysteinaemia and may provide another simple mode of treatment.[75]

Vermeulen *et al.*[76] in a small, short-term trial of vitamin therapy, could only show an improvement in coronary artery risk, as assessed by exercise electrocardiography tests, with HCy lowering treatment. More recently, his group has reported results from a large prospective trial of hyperhomocysteinaemic patients presenting with premature PVD or cerebrovascular disease (before the age of 56 years). When treated with folic acid and B_6, these patients demonstrated a similar risk of future cardiovascular events as normohomocysteinaemic controls.[77,78]

The results from large-scale, long-term randomised controlled trials of such regimens in people at high risk from cardiovascular events and those undergoing therapeutic intervention are currently awaited.

Conclusions

Restenosis is a common and serious problem encountered after arterial bypass or endoluminal treatment of atherosclerotic arterial disease and any effective treatment could potentially have wide applications in both coronary and peripheral vascular disease. HCy, if proven to be a true primary risk factor, would be an attractive target for therapy since it is both easily measured and easily treated. To date the majority of epidemiological studies have confirmed that elevated levels of HCy are associated with PVD, though the association with the development of MIH is still tenuous. Animal and *in vitro* studies have demonstrated potential mechanisms by which HCy could produce endothelial injury, smooth muscle cell activation and other aspects of the pathophysiology of MIH, and support the hypothesis that it is an important risk factor for MIH. Though few prospective studies and no randomised trials of HCy lowering therapy have yet been published. Such studies are needed before therapy to lower HCy can be widely recommended.

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