Homocystinuria: what about mild hyperhomocysteinaemia?

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Atherosclerotic disease, notably coronary artery disease, remains the major cause of death in the western world. This increased risk of mortality from cardiovascular disease cannot be fully explained by traditional risk factors such as hyperlipoproteinemia, smoking, hypertension and male sex. Since the last decade, mildly elevated homocysteine levels have also been recognised as a serious risk factor in the development of atherosclerotic disease and thromboembolism.

Severe hyperhomocysteinaemia, such as in homocystinuria due to cystathionine synthase deficiency, is inherited as an autosomal recessive trait and may lead to occlusive arterial disease and thromboembolism even in early infancy. From a collaborative study on more than 600 homozygous patients, Mudd et al observed that there was a 50% chance for untreated patients to suffer from a vascular event before the age of 30 years. In line with this observation, the hypothesis has been put forward that even mild hyperhomocysteinaemia may predispose for arterial occlusive disease, even at a young age. Indeed, case-controlled, cross-sectional, and prospective epidemiological studies have shown that mildly elevated homocysteine levels are associated with an increased risk of arterial occlusive disease.

This relationship has been reported for both fasting homocysteine levels and for hyperhomocysteinaemia unmasked by means of a methionine loading test in which homocysteine metabolism is stressed with an oral dose of L-methionine (0.1 g/kg body weight). Whether fasting or post-methionine plasma homocysteine level is the better predictor of vascular disease has not been clarified.

It has been shown that safe doses of vitamins can induce normalisation of such increased homocysteine blood levels and may ameliorate endothelial function in mildly hyperhomocysteinaemic vascular patients. In conclusion, mild hyperhomocysteinaemia has been recognised as an important risk factor for atherosclerotic disease, thus providing the rationale for large-scale screening and intervention with innocuous vitamins in patients with elevated homocysteine levels. Relevant aspects of this unconventional risk factor are briefly discussed in this paper.

Metabolism and aetiology of hyperhomocysteinaemia

The essential amino acid methionine, which is present in protein of animal origin, is the only source of homocysteine in man. Normally, homocysteine is rapidly catabolised by transsulphuration into cysteine or is remethylated to methionine (figure 1). Cystathionine synthase catalyses the first step in transsulphuration with vitamin B6 in its active form, pyridoxal phosphate, as cofactor. In many body tissues remethylation by the folate- and vitamin B12-dependent enzyme N5-methyltetrahydrofolate-homocysteine methyltransferase is operational and in the liver homocysteine is also remethylated by betaine (trimethylglycine), formed from choline.

Plasma homocysteine is the sum of homocysteine, whether free or bound to proteins, and the homocysteinyl moieties of the disulphides homocystine and cysteine-homocysteine. Hyperhomocysteinaemia refers to levels of such 'total' homocysteine level in the blood, plasma or serum. Epidemiological studies strongly suggest a graded response rather than a threshold effect of homocysteine levels, but for practical reasons the following classification has been made: moderate, intermediate and severe fasting hyperhomocysteinaemia, with levels between 16 and 30, 31 and 100, and more than 100 μmol/l, respectively. In diagnosing hyperhomocysteinaemia in the individual patient it is preferable to perform a methionine loading test, as stated later. The generally used cut-off points for post-load hyperhomocysteinaemia are sex specific, i.e., in men ≥4, in premenopausal women ≥51, and in postmenopausal women ≥69 μmol/l, respectively.

Severe hyperhomocysteinaemia, also known as homocystinuria, is an
abnormal biochemical phenotype (figure 2) associated with deficient activity of one of several enzymes in methionine metabolism, notably cystathionine synthase, 5,10-methylenetetrahydrofolate reductase, and disorders of methionine synthase activity due to a defect in synthesis of methylcobalamin (figure 1). Disorders of cystathionine synthase impair homocysteine flux through the transsulfuration pathway; disorders of the other two enzymes affect homocysteine remethylation to methionine.¹

Mild hyperhomocysteinemia, either in the fasting state or after a standardised methionine load, can be the consequence of intermediate deficiency (about 50% rest activity) of one of the involved enzymes as mentioned above. Such an intermediate effect is known to cause mild hyperhomocysteinemia in heterozygotes for cystathionine synthase deficiency,¹²,¹³ but not in carriers of 5,10-methylenetetrahydrofolate reductase deficiency.¹² Recently the existence of an inherited mutant variant of 5,10-methylenetetrahydrofolate reductase characterised by thermolability has been demonstrated (box 1). Heterozygotes for this mutation can show mild hyperhomocysteinemia although it is not obligatory,¹⁴ whereas heterozygotes are normohomocysteinaemic.¹⁴ Compound heterozygotes of both reductase mutations are also hyperhomocysteinaemic in the majority of cases.¹⁴

The variation in individual plasma homocysteine levels is caused not only by genetic but also by environmental factors. Homocysteine plasma levels rise with decreasing folate and vitamin B₁₂ intakes and blood levels.¹⁶ Dietary vitamin B₁₂ deficiency has been shown to increase the excretion of homocysteine in the urine after a methionine load.¹⁷ An effect on the fasting homocysteine blood level, however, could not be demonstrated by such a deficiency,¹⁸ and in another study, low plasma vitamin B₁₂ levels were not related to homocysteine concentrations before and after methionine loading (box 1).¹⁹

Plasma homocysteine is increased in patients with renal failure. This has been hypothesised to contribute to the high incidence of occlusive arterial disease in these patients. The increase of plasma homocysteine is moderate to marked (up to 50 μmol/l) and is positively correlated with serum creatinine.²⁰ Impaired methionine clearance after loading with the amino acid is reported in chronic liver disease and hepatic insufficiency and supposedly will affect homocysteine concentrations in the blood, although data on such measurements are not available.²⁰

Increased homocysteine levels have been reported in type 1 diabetic mellitus patients with serum creatinine levels above 115 μmol/l due to diabetic nephropathy, but not in those with minimal nephropathy.²¹ In addition, a lack of association between plasma homocysteine and microangiopathy was recently reported in patients with type 1 diabetes mellitus, however, patients with clinical signs of nephropathy had higher plasma homocysteine levels.²² In conclusion, type 1 diabetes is not per se associated with increased plasma homocysteine levels but, if homocysteine accumulates due to advanced nephropathy, it may
contribute to the accelerated development of macroangiopathy in diabetics. These findings should therefore be considered in the context of a positive relation between homocysteine and creatinine.

The intra-individual day-to-day variation in fasting homocysteine levels is rather wide, up to 25%. One of the causes of this variation could be dietary changes during the day preceding the fasting samplings because it has been shown that a protein-rich meal may affect plasma homocysteine levels for at least eight hours. A post-methionine-load homocysteine determination, four to six hours after the methionine intake, will have a distance in time from the last protein-containing meal of at least 16 hours and during the test itself the protocol inhibits protein intake. Therefore, less intra-individual variation in post-methionine plasma homocysteine levels might be expected (box 1).

The antifolate drug methotrexate, folate-deficiency-inducing anti-epileptics, and nitrous oxide, which inactivates vitamin B12, will increase plasma homocysteine (box 1). Recently, the modulation of plasma homocysteine by these and various other pharmacological agents, such as penicillamine and oestrogens, has been extensively reviewed.

**Mild hyperhomocysteinaemia and vascular disease**

In 1976, Wilcken and Wilcken were the first to publish results suggesting that even mild hyperhomocysteinaemia could have a possible role in the pathogenesis of coronary artery disease. They showed that about 30% of young patients with angiographically proven coronary artery disease demonstrated mild hyperhomocysteinaemia, four hours after a methionine load. This unique finding suggested an important role for even mild hyperhomocysteinaemia in the pathogenesis of vascular disease at a young age. Since then, numerous studies have shown that mild hyperhomocysteinaemia is a frequent finding amongst vascular patients. In 1992 pooled data revealed a prevalence of 32% in patients with peripheral vascular disease, 24% in patients with cerebrovascular disease, and 21% in patients with coronary artery disease. From pooled data an odds ratio of 13.0 (95% CI 5.9–28.1) could be calculated as an estimate of the relative cardiovascular risk in subjects with an abnormal response to methionine loading compared to normal responders. The recently published data by van den Berg et al and Franken et al are in line with the finding of high prevalences of mild post-load hyperhomocysteinaemia amongst vascular patients (box 2). Recently, prospective studies have indeed confirmed that mild hyperhomocysteinaemia in men is associated with an increased incidence of myocardial infarction and, possibly, of stroke. In addition, a dose-response relationship is supported by the fact that hyperhomocysteinaemia is reported to be associated with the severity of peripheral arterial disease and with the number of stenosed coronary vessels in arteriosclerotic patients. Malinow et al observed a graded increase in the prevalence of carotid artery intimal-medial thickening with increasing random homocysteine levels in asymptomatic subjects, which was more pronounced in women. Selhub et al reported a relationship between random homocysteine levels and the prevalence of carotid artery stenosis. Taken together, the epidemiologic evidence may indicate a graded effect of plasma homocysteine levels, as in hypercholesterolaemia, rather than a threshold effect.

It has recently been shown that mild hyperhomocysteinaemia is a strong risk factor for recurrent venous thrombosis and can lead to a two-fold or three-fold increase in risk. Possible relationships between plasma homocysteine and conventional risk factors for vascular disease have been studied but no such relation was established for tobacco smoking, hypertension, serum lipids, or diabetes mellitus. Therefore, so far, hyperhomocysteinaemia seems to be an independent risk factor for cardiovascular disease.

At this stage, it is unresolved which is the most sensitive indicator of excess cardiovascular risk, either the fasting, the post-load homocysteine level, or both (box 3). Identification of patients at risk simply on the basis of their fasting homocysteine level might be insufficient because, depending on the cut-off point, about 40–54% of patients with high post-methionine homocysteine have a normal fasting level. The latter finding, moreover, suggests the existence of multiple underlying metabolic deficits, because fasting and post-methionine homocysteine levels are thought to be determined by different pathways, i.e., remethylation and transsulphuration of homocysteine, respectively. A serious argument against the use of the post-methionine homocysteine level as the criterion of hyperhomocysteinaemia is the fact that the methionine loading test is too laborious and expensive to include in the design of epidemiological studies with large numbers of participants. Even in diagnostic procedures in individual vascular patients, the practical concerns and high costs of establish-
ing post-methionine homocysteine levels suggest that this test is not feasible as a routine screening procedure. If it is to become so, more effort is needed to standardise the sampling of fasting homocysteine levels and more attention should be paid to the food intake on the day preceding the blood sampling, to minimise the wide intra-individual daily variation of these levels, as mentioned before.

Pathogenesis of vascular disease in mild hyperhomocysteinaemia

Homocysteine, an amino acid with a free sulphhydril group, is generally held to be atherogenic and thrombogenic, although the exact pathophysiological sequence has not been clarified. Homocysteine is thought to damage endothelial cells by several mechanisms, eg, generation of hydrogen peroxide, oxidation of low-density lipoproteins, and depletion of nitric oxide-mediated detoxification of homocysteine. In studies in animals, high homocysteine levels have been shown to induce endothelial cell injury. In vitro studies indicate that cultured endothelial cells from obligate heterozygotes for homocystinuria are more susceptible to homocysteine-mediated injury than normal cells. Another potential mechanism, recently described by Tsai et al is that homocysteine induces the proliferation of smooth muscle cells, a key feature of atherogenesis. It has also been reported that homocysteine inhibits the expression of thrombomodulin on the surface of the endothelial cell, leading to decreased protein C activation and thus, possibly, contributing to the development of thrombosis in hyperhomocysteinemic patients.

Although data in humans are scarce, van den Berg et al showed in hyperhomocysteinaemic patients with lower extremity occlusive atherosclerotic disease that endothelial dysfunction, as estimated by increased plasma von Willebrand factor concentrations, was ameliorated by treatment of hyperhomocysteinaemia with folic acid and vitamin B<sub>12</sub>.

In conclusion, abnormalities of endothelial cells, platelets, clotting factors, serum lipids or disorders in the complex interaction of these factors have been held responsible for the vascular damage and thrombogenesis. A survey of all proposed hypotheses has been presented elsewhere (box 4).

The cause of mild hyperhomocysteinaemia in vascular patients

Analogous to the first observation by Sardharwalla et al of mildly elevated homocysteine levels in obligate heterozygotes for cystathionine synthase deficiency, it has been proposed that such hyperhomocysteinemia detected in vascular patients also originates from heterozygosity for this specific enzyme defect. In the two reports presenting determinations of cystathionine synthase activity in cultured fibroblasts from hyperhomocysteinemic vascular patients, an intermediate enzyme deficiency was indeed found. In recent years, however, it has become increasingly clear that the identification of patients with vascular disease as heterozygotes for cystathionine synthase deficiency is not convincing. More recent determinations of enzyme assays in hyperhomocysteinemic vascular patients show that cystathionine synthase activity is deficient only in sporadic cases and wide variability in activity in cultured fibroblasts may occur, even in healthy persons and recently it has been shown that heterozygous carriers and homocystinuric patients were free from inactivating mutations.

The role of the cystathionine synthase gene may have been overestimated in the past and, therefore, the re-methylation pathway has gained more interest in recent years. Recently, Rozen's group cloned the gene responsible for the enzyme methylenetetrahydrofolate reductase, which has permitted mutational analysis in this gene, facilitating the evaluation of the re-methylation pathway in the aetiology of hyperhomocysteinaemia. Thermolability of 5,10 methylenetetrahydrofolate reductase proves to be a far more common genetic defect among these patients. Homozygotes for this mutation have been detected in 5% of the general population and in up to 17% of 212 coronary patients. Recently, a common mutation in 5,10-methylenetetrahydrofolate reductase has been identified, which in the heterozygous or homozygous state correlates with reduced enzyme activity and decreased thermostability in lymphocyte extracts. In addition, individuals homozygous for the mutation have significantly elevated plasma homocysteine levels, both in the fasting state and after methionine loading. Notwithstanding the occurrence of thermostable enzyme in 28% of some groups of hyperhomocysteinaemic vascular patients, this prevalence still does not seem high enough to predict a predominant role of this defect among possible causes of mild hyperhomocysteinaemia in vascular patients.

**Box 4**

Atherogenetic mechanisms of homocysteine

- damaging endothelial cells by generation of hydrogen peroxide, oxidation of low-density lipoprotein, and depletion of nitric oxide
- proliferation of smooth muscle cells
- coagulation/fibrinolysis interaction: inhibiting expression of thrombomodulin
Mild hyperhomocysteinaemia and vascular disease

<table>
<thead>
<tr>
<th>Treatment</th>
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<tr>
<td><strong>In fasting homocysteine</strong></td>
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<tr>
<td>folic acid:</td>
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<tr>
<td>2.5 mg daily: 37% reduction</td>
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<tr>
<td>5 mg daily: 50% reduction</td>
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<tr>
<td>10 mg daily: 50% reduction</td>
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<tr>
<td>vitamin B6: no effect</td>
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<tr>
<td>vitamin B12: only modest effect (−15%), but should be added to avoid folic acid refractoriness and deterioration of neuropathy in vitamin B12-deficient cases. A sufficient dose is 0.4 mg daily</td>
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<tr>
<td><strong>In post-methionine hyperhomocysteinaemia</strong></td>
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<tr>
<td>vitamin B6, 100 mg + folic acid, 5 mg daily: 50% reduction</td>
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<tr>
<td>vitamin B6, 100 to 250 mg daily: 40% reduction</td>
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<tr>
<td>folic acid (n = 6 patients only): 45% reduction</td>
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<tr>
<td>(lower doses of vitamin B6 and/or folic acid have not been studied)</td>
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<tr>
<td><strong>To lower fasting homocysteine levels</strong></td>
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<tr>
<td>0.65 mg folic acid plus 0.4 mg cyanocobalamin</td>
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<tr>
<td><strong>To lower post-methionine homocysteine levels</strong></td>
</tr>
<tr>
<td>100 mg vitamin B6 plus 5 mg folic acid</td>
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**Homocysteine-lowering interventions**

**FASTING HOMOCYSTEINE LEVELS**
From pooled data, it can be estimated that it is possible to induce a reduction of about 40% of mildly elevated fasting plasma homocysteine levels by the use of doses of folic acid as low as 0.65 mg daily. A lower dose has not been studied. A dose of 2.5 mg daily had the same effect as 0.65 mg, 5 mg daily resulted in a slightly larger decrease of about 50%, and increasing the dose up to 10 mg daily had no extra effect. Supplementation of vitamin B6 did not affect fasting homocysteine levels significantly. Vitamin B12 in an oral dose of 0.4 mg cyanocobalamin daily has only a modest effect but is added to folic acid therapy mainly to avoid folic-acid refractoriness in the case of vitamin B12 deficiency and to prevent the development of neuropathy due to unrecognised pernicious anaemia (box 5). In summary, the prescription of 0.65 mg folic acid plus 0.4 mg cyanocobalamin daily is sufficient to lower a mild fasting hyperhomocysteinaemia by about 50%.

**POST-METHIONINE HOMOCYSTEINE LEVELS**
Available data on the effect of various homocysteine-lowering regimens upon mildly elevated post-methionine homocysteine levels show that the use of 100 mg vitamin B6 plus 5 mg folic acid decreases homocysteine levels by about 50%. Vitamin B6 as a single agent in doses from 100 to 250 mg daily resulted in a slightly lower reduction of about 40%. Folic acid as the sole therapy has been studied in only six patients so far and showed a 45% decrease in these patients. The efficacy of lower doses of vitamin B6 and/or folic acid as a single or combined therapy has not been explored. Remarkably, only the combination of vitamin B6 plus folic acid resulted in normalisation of post-methionine homocysteine levels in 90% or more of the treated patients, whereas the respective single treatments did much less, ie, about 50% (box 5). In summary, 100 mg vitamin B6 and 5 mg folic acid daily should be prescribed for the treatment of post-methionine hyperhomocysteinaemia.

**Conclusions**

The prevalence of mild hyperhomocysteinaemia in young patients with arterial occlusive disease is high and hyperhomocysteinaemia has to be accepted as a serious risk factor for vascular disease. Future studies will hopefully elucidate in which way atherosclerosis and thromboembolic events are induced in patients with hyperhomocysteinaemia. Furthermore, simple and inexpensive therapy with innocuous vitamins can normalise homocysteine metabolism, as assessed by the homocysteine plasma level before and after methionine loading, in virtually all these patients. Intervention studies are needed to clarify if such treatment will also reduce morbidity and mortality. The demonstration of a clinical benefit of homocysteine-lowering interventions would be required to
Learning points

- mild hyperhomocysteinemia is a risk factor for atherosclerotic disease and venous thrombosis
- a methionine loading test is recommended for the diagnosis of hyperhomocysteinemia in the individual patient
- in the case of an abnormal test result, treatment with vitamins involved in homocysteine metabolism should be considered

Box 6

justify routine screening for this risk factor on a large scale. Meanwhile, to clarify the individual patient’s risk of developing atherosclerosis or thromboembolic events, it is advisable to perform a methionine loading test for the screening of hyperhomocysteinemia, at least in those cases in which conventional risk factors are absent (box 6).