Heart

Editorial

Homocysteine, B vitamins, and risk of cardiovascular disease

Homocysteine is a sulphur containing amino acid that plays an important role in methionine and folate metabolism.[1] By receiving a methyl group from 5'-methyltetrahydrofolate, it is reconverted to methionine (fig 1), which is essential for many biochemical reactions critical to the formation of protein, nucleic acids, and creatinine. Reconversion of homocysteine to methionine also contributes to the maintenance of intracellular stores of tetrahydrofolate.

Homocysteine has become a target for many basic and clinical investigators because of a clinical syndrome described almost 40 years ago.[2] [3] The syndrome, homocystinuria, is an autosomal recessive disorder characterised by abnormalities of the long bones, ocular lens dislocation, mental retardation, and aggressive vascular disease, in particular venous thromboembolism. The underlying enzymological abnormality, a deficit of cystathionine β synthase, results in impairment of homocysteine transsulfuration. As a consequence, the concentrations of the amino acid in plasma may rise 20-fold from the normative range of 5–15 µmol/l. About 10 years after the discovery of homocystinuria it was hypothesised by McCully[4] that high plasma homocysteine might be causally related to the vascular complications of the disease. In 1976, Wilcken and Wilcken[5] studied patients without homocystinuria but with angiographically proved coronary artery disease. Using the methionine loading test, in which 0.1 mg/kg body weight of this amino acid is administered orally, they found that the prevalence of high circulating homocysteine concentrations, or hyperhomocysteinaemia, was higher than in normal controls. They concluded that people with a genetic susceptibility, namely carriers of the genetic mutation for cystathionine β synthase deficiency, might be at greater risk of coronary artery disease.

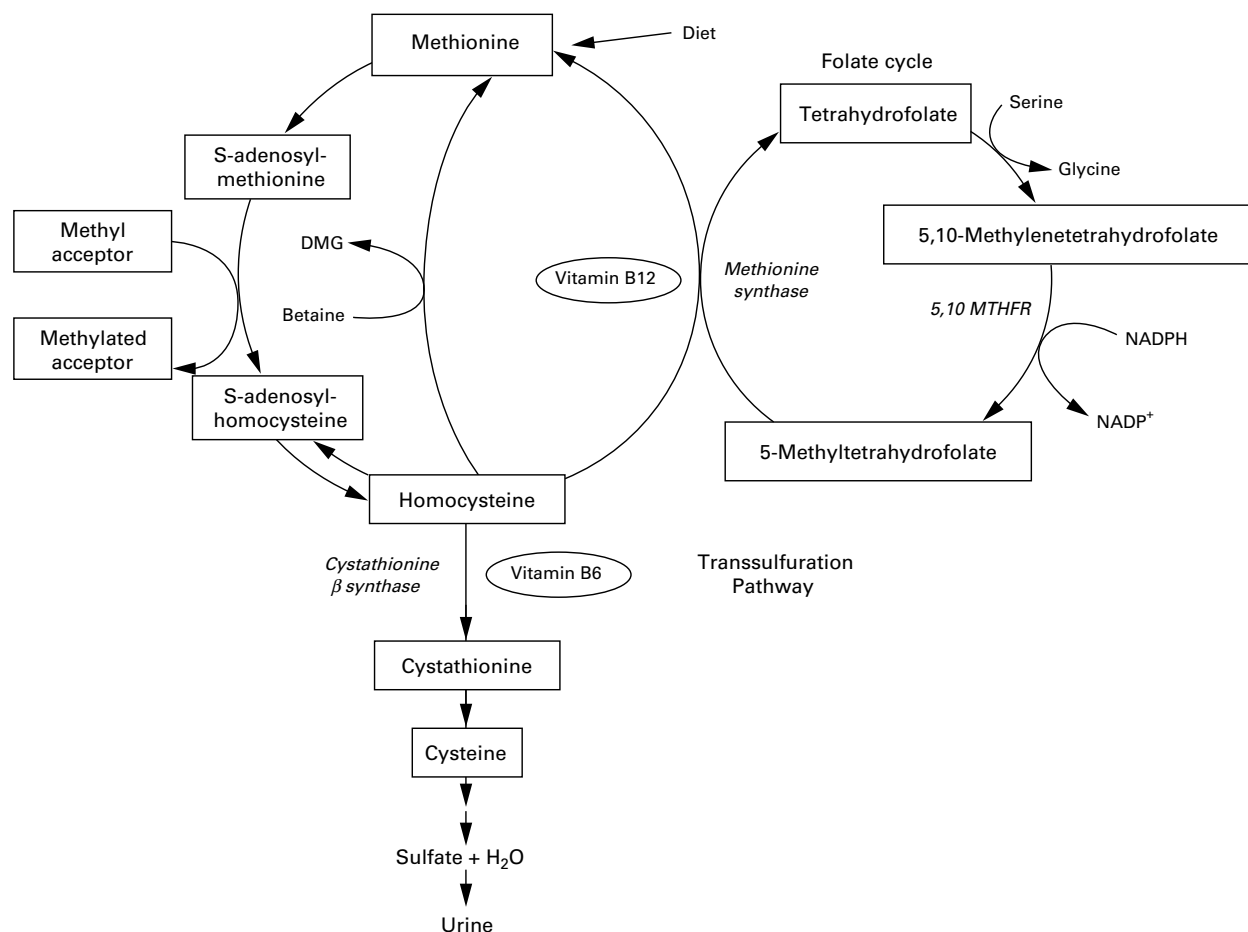*Figure 1   Pathways for the metabolism of homocysteine. Normal transsulfuration requires cystathionine β synthase with vitamin B6 as cofactor. Remethylation requires 5, 10-methylenetetrahydrofolate reductase and methionine synthase. The latter requires folate as cosubstrate and vitamin B12 (cobalamin) as cofactor. An alternative remethylation pathway also exists using the cobalamin independent betaine–homocysteine methyltransferase.*

Table 1   Causes of hyperhomocysteinaemia

Male sex
Age
Menopause
Lifestyle factors including smoking, heavy intake of coffee, and alcohol

Genetic
   Cystathionine β synthase deficiency (the cause of classic homocystinuria)
   5,10-Methylenetetrahydrofolate reductase deficiency (rare) or
      thermolability (common)
   Methionine synthase deficiency (rare)

Nutritional disorders
   Folate deficiency
   Vitamin B12 deficiency
   Vitamin B6 deficiency

Systemic disease
   Renal failure
   Malignant diseases
   Psoriasis
   Hypothyroidism

Drugs
   Cholestyramine and colestipol (decrease cobalamin and folate absorption)
   Methotrexate (inhibits dihydrofolate reductase)
   Phenytoin and carbamazepine (antagonise folate)
   Nitrous oxide (inactivates methionine synthase)
   Niacin and theophylline (decrease pyridoxal kinase)
   Androgens (increase muscle mass and serum creatinine)
   Cyclosporin A (decreases renal function)
   Metformin (may decrease cobalamin absorption)
   Fibric acid derivatives (may interfere with renal function)

Hyperhomocysteinaemia: result of a gene–nutrient interaction

For some years, attention focused on the role of heterozygosity for homocystinuria[6] as a possible cause of the high homocysteine concentrations that are seen in up to 30–40% of patients with coronary artery disease.[1] This cause is now felt unlikely. Indeed, a glance at the biochemical pathways of homocysteine metabolism shows that abnormalities of other critical enzymes and several cofactors could also lead to high homocysteine concentrations.[1] For example, transsulfuration of homocysteine also requires vitamin B6, an essential cofactor for cystathionine β synthase. It is no surprise, therefore, that plasma concentrations of this vitamin correlate negatively with those of homocysteine. In addition, the remethylation of homocysteine, which results in the reconstitution of methionine and tetrahydrofolate, also requires the enzymes methionine synthase and methylenetetrahydrofolate reductase (MTHFR). Deficiencies of these enzymes, although rare, have been described. Predictably, they are associated with hyperhomocysteinaemia and notably they are also associated with vascular disease. Two relatively common variant polymorphisms in the gene coding for MTHFR have been described.[1 7] One of these, the C677T mutation, may also be associated with hyperhomocysteinaemia especially in the presence of lower folate concentrations. Its relation to coronary artery disease remains controversial although a recent meta-analysis has shown the same prevalence in patients with cardiovascular disease as in controls.[8] Methionine synthase requires cobalamin (vitamin B12) and folate as cosubstrate, and deficiencies of both of these vitamins are well known causes of hyperhomocysteinaemia (table 1).[1]

Other factors affecting plasma homocysteine

AGE, SEX, AND LIFESTYLE

Homocysteine concentrations rise with age in both sexes. Women in general have lower concentrations than men, and concentrations rise after menopause. Cigarette smoking, a high intake of caffeine or alcohol, and a sedentary lifestyle are associated with raised homocysteine.[1] At a population level, however, the most important causes are probably lower concentrations of folic acid and vitamins B12 and B6.[9]

SYSTEMIC ILLNESSES

Creatinine correlates strongly with plasma homocysteine. In end stage renal disease homocysteine concentrations increase two- to threefold.[10] Values are higher in patients undergoing haemodialysis compared to peritoneal dialysis. The exact mechanisms for the raised levels of the amino acid seen in these patients remain unclear but reduced systemic clearance of homocysteine, lower circulating folate, and folate inhibition are probably major determinants. Homocysteine may also rise with various cancers, hypothyroidism,[1] inflammatory bowel disease,[11] and following organ transplantation.[12]

EFFECTS OF MEDICATION AND VITAMIN SUPPLEMENTATION

Predictably, increased concentrations of homocysteine accompany the use of inhibitors of folate such as methotrexate and carbamazepine, or antagonists of folate absorption such as colestipol and cholestyramine.[1] Nitrous oxide acts as a cobalamin antagonist. Theophylline and niacin may result in vitamin B6 deficiency.[13 14] Cyclosporin may impair renal function and it has been associated with hyperhomocysteinaemia. Homocysteine concentrations fall with the use of B vitamins: folic acid, vitamin B12, and betaine encourage remethylation whereas vitamin B6 promotes increased transsulfuration.[1] The aminothiols penicillamine and acetylcysteine may also reduce plasma homocysteine as may exogenous oestrogen.[1]

Homocysteine as a risk factor for vascular disease

Since the early study of Wilcken and Wilcken[5] many case control studies have shown that homocysteine is an independent risk factor for atherosclerotic vascular disease and for venous thrombosis.[1] Broadly similar findings are found irrespective of whether the homocysteine is measured in the fasting state or following the methionine loading test. For example, in a European multicentre investigation of 750 cases and a similar number of controls, a high homocysteine concentration conferred a risk equal to that of hypercholesterolaemia, smoking or hypertension.[15] Some, but not all, prospective (or nested case control) studies have confirmed these findings. In the multiple risk factor intervention trial (MRFIT), for example, the homocysteine concentrations in serum samples from men who subsequently suffered a myocardial infarction were no different from those in controls.[16] In another recent prospective study by Folsom et al higher homocysteine concentrations added to the risk of incident coronary artery disease in women but not in men.[17] In patients with coronary disease, higher homocysteine concentrations are related to lower circulating B vitamin concentrations. Indeed, in one study, as the concentration of folate fell, the levels of both homocysteine and cardiovascular risk rose.[18] Similarly, there is an increased vascular risk associated with lower levels of vitamin B6 both in case control[19] and prospective studies.[17] Hyperhomocysteinaemia has also been associated with increased atherothrombotic risk in end stage renal disease[10] and systemic lupus erythematosus.[20]

Possible pathophysiological mechanism of vascular injury

IN VITRO STUDIES

Clinical and experimental studies suggest that high homocysteine concentrations may cause the atherogenic and thrombotic tendencies of homocystinuric and hyperhomocysteinaemic patients.[1 21] A single discrete mechanism, however, remains unproved. Homocysteine may have adverse effects on platelet function, clotting factors and may also promote vascular smooth muscle cell migration and proliferation.[1 22] It may also exert important negative influences on endothelial function and result in either prooxidant or prothrombotic effects. For example, homocysteine auto-oxidises readily and forms potentially noxious hydrogen peroxide and superoxide anion in the process.[21] Endothelium derived nitric oxide may protect

against this as, in its presence, homocysteine is converted to S-nitrosohomocysteine preventing the generation of hydrogen peroxide.[23] S-nitrosothiols also have potent vasodilatory and antiplatelet activities. Other endothelial cytoprotective forces include glutathione, which maintains intracellular sulfhydryl groups including homocysteine in reduced form, and glutathione peroxidase, a naturally occurring intracellular buffer that catalyses the reduction of hydrogen peroxides and lipid peroxides to their respective alcohols.[21] Chronic hyperhomocysteinaemia may, however, overwhelm these endothelial defence mechanisms resulting in vascular damage. Alternatively, the free thiol group of homocysteine may directly damage endothelial cells. Previous in vitro cell culture experiments have shown such a direct cytotoxic effect of homocysteine on endothelial cells.[21]

Whatever the underlying cause of the insult, endothelial injury may convert the normally antithrombotic endothelial surface to one which favours thrombosis. Activation of factor V, reduced protein C activation, inactivation of thrombomodulin, decreased binding of antithrombin 3, and impaired secretion of von Willebrand factor have all been reported as possible adverse effects of raised homocysteine levels. Decreased t-PA binding or endothelial expression of anticoagulant heparan sulfate activity are other possibilities.[1]

ANIMAL AND HUMAN STUDIES

In one study, mini-pigs were fed a methionine rich diet which caused hyperhomocysteinaemia and the animals subsequently developed arterial elastic alterations.[24] Diet induced hyperhomocysteinaemia has also been produced in the cynomolgus monkey.[25] This was followed by impaired endothelial dependent vasodilation and decreased thrombomodulin dependent activation of protein C. Furthermore, the levels of homocysteine causing altered vascular function occurred at concentrations of homocysteine associated with atherosclerosis in humans. In observational studies in human subjects, raised homocysteine is also associated with impaired endothelial dependent vasodilation suggesting similar adverse effects on nitric oxide in man.[26]

Recently, Chambers *et al* studied brachial artery diameter responses to endothelium dependent hyperaemic flow using high resolution ultrasound in 17 healthy subjects before and after the methionine loading test. Plasma homocysteine increased, and flow mediated dilatation fell at 0, 2, and 4 hours. There was an inverse linear relation between homocysteine concentration and flow mediated dilatation. Pretreatment with vitamin C did not blunt the rise in homocysteine concentrations but it did improve the reduction in flow mediated dilatation, supporting the notion that the adverse effects of homocysteine on endothelial cells are mediated through oxidative stress mechanisms.[27] It is clear that there is no one unifying hypothesis of the mechanism, if any, of homocysteine induced vascular damage although at present the endothelium is the most likely candidate.

Treatment and trials

FOLIC ACID SUPPLEMENTS

Folic acid is the treatment of choice for reduction of high homocysteine concentrations. Most patients only require doses of 0.4 mg, the current recommended dietary allowance for this vitamin in the USA. Such amounts are inexpensive, highly effective, and safe. A meta-analysis of 12 randomised controlled trials involving 1114 patients showed that daily supplementation with 0.5–5 mg of folic acid and 0.5 mg of vitamin B12 decreases homocysteine concentrations by a quarter to a third.[28] In another study, the effects of daily doses of 0.1, 0.2, and 0.4 mg folic acid were compared; and 0.2 mg was as effective as 0.4 mg.[29] Although usually

innocuous, it should be emphasised that the use of folic acid alone may predispose to neurological damage in those who are also deficient in vitamin B12. This is of particular importance in the elderly in whom it may be prudent to check vitamin B12 levels if long term folic acid treatment is considered. In some clinical situations, dose requirements may rise. In patients with end stage renal disease, for example, folic acid doses as high as 15 mg/day have been used[10] and may still not normalise the raised homocysteine concentrations typically seen in this clinical condition. Larger doses than 0.4 mg may also be needed in patients on drugs that antagonise the actions of folate.

These homocysteine lowering effects of folic acid may translate into favourable clinical outcomes. Woo *et al* have shown that folic acid supplementation may improve endothelium dependent vasdilatation.[30] Similar findings have been reported in patients with familial hypercholesterolaemia using 5-methyltetrahydrofolate, the active form of folic acid.[31] Even more provocative are the findings of Peterson and Spence who, in a small uncontrolled study, showed that supplemental folic acid may decrease the progression of carotid atherosclerosis.[32]

Clinical trials

Several large secondary prevention trials using folic acid either alone or combined with vitamins B6 or B12 are now in progress in patients with vascular diseases. In the USA, the vitamin intervention for stroke prevention (VISP) study is assessing the effect of B vitamins on the recurrence of stroke in patients with established cerebrovascular disease. In Scandinavia, the Norwegian Study of homocysteine lowering with B vitamins in myocardial infarction (NORVIT) and the western Norwegian study on the effect of homocysteine reduction with B vitamins in patients with angiographically verified coronary artery disease (WENBIT) are also underway. The study of the effectiveness of additional reductions in cholesterol and homocysteine (SEARCH) is in progress in the UK and the prevention with a combined inhibitor and folate in coronary heart disease (PACIFIC) trial is ongoing in Australia. Other studies in similar patient populations and in patients with end stage renal disease are being performed in Europe, Canada, and the USA, and the results are expected in the next several years. Hopefully, they will determine if the use of B vitamin supplements leads to improved outcomes in these patients.

Many important questions remain—for example, is a high circulating homocysteine concentration causal in the pathogenesis of coronary disease, and other atherosclerotic and thrombotic vascular diseases, or is it merely an epiphenomenon? Is the high homocysteine level a reflection of lower B vitamin status which is itself directly (or indirectly) linked to atherosclerosis? Or is it a reflection of diminished renal function so often seen in patients with vascular disease?[8] What, if any, is the mechanism by which homocysteine may induce atherosclerosis? In other clinical circumstances such as following organ transplantation, or in the presence of hypothyroidism or inflammatory bowel disease, does high homocysteine predict increased vascular risk?

For the clinician in practice, the routine measurement of homocysteine concentrations, although advocated by some, remains speculative until the results of the current intervention trials become known.[33] Similarly, the use of pills containing low dose B vitamins as a primary or secondary preventive measure against vascular disease remains unproved, although they are definitely cheap, probably innocuous, and may help prevent acute vascular episodes.

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STAMPS IN CARDIOLOGY

Health campaigns

Morocco issued a single stamp in 1971 to mark the European and North African Heart Week. The unusual design depicts the heart within a horse. The 90 centavos Argentine Republic stamp is from 1972 commemorating World Health Day. The same event was featured in the 10 rial stamp from Iran, which also denotes the Iranian Society of Cardiology.

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