Homocysteine-induced thrombosis

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ABSTRACT

The connection between homocysteine and thrombosis was identified approximately 25 years ago when it was reported that people with a rare condition called homocystinuria accumulated homocysteine in the blood and excreted it in the urine. Recent studies provide overriding evidence to suggest that elevated blood homocysteine levels can cause thrombosis and some 10–20% of coronary heart disease cases have been linked to elevated homocysteine levels. Factors such as hereditary predisposition, ethnic origin, gender, age and diet affect homocysteine level but the mechanisms by which homocysteine causes thrombosis are largely unknown. Further information on the mechanisms involved has emerged in the last few years and this essay elucidates these developments.

KEY WORDS: Homocysteine. Thrombosis.

Introduction

In 1932 the American biochemist DuVigneaud[1] discovered a new amino acid by removing the methyl group in methionine. This compound, termed homocysteine, has the same functional groups (sulfhydryl, amino and carboxyl) as the amino acid cysteine but it contains one additional carbon atom.

Homocysteine can be converted to methionine by the transfer of methyl groups from compounds such as choline and betaine – a process known as transmethylation. DuVigneaud also discovered that homocysteine can be converted to cysteine, through the intermediate formation of cystathionine, during metabolism. However, homocysteine remained a clinically insignificant compound for almost two decades until the mid-1970s when McCully and Wilson[2] reported the presence of arteriosclerotic plaques in the aorta and arteries of rabbits given homocysteine. The work of Harker *et al*.[3] also showed that homocysteine induced endothelial injury and that arteriosclerosis was prevalent in baboons.

In 1962, Irish investigators screened the urine of children with mental retardation using paper and column chromatography to detect the presence of amino acids.[4] Several of these patients were found to have homocysteine in their urine (homocystinuria) and showed a tendency to develop thrombi in arteries and veins. Cases of homocystinuria were found almost simultaneously in the USA.[5] It was also discovered that cystathionine synthase, a vitamin B_6–dependent enzyme, is deficient in cases of homocystinuria and that vitamin B_6 therapy proved effective as a remedy.[5]

An extensive study[6] carried out recently investigated the blood homocysteine levels of 131 patients with severe blockages in two coronary arteries, 88 patients with moderate blockage in one coronary artery, and another group of healthy individuals without heart disease. A linear relationship between blood homocysteine levels and severity of coronary blockage was revealed. For every 10% rise in homocysteine level, there was a 10% increase in the risk of developing severe coronary heart disease.

Other studies[7-10] published in the last two decades also highlight the importance of regulating homocysteine levels to reduce the risk of thrombosis. For example, post-menopausal women with elevated homocysteine levels showed a higher incidence of coronary heart disease[7] and homocysteine levels were much higher in people who developed vein clots.[8] Thus, the evidence against elevated homocysteine as a causative agent of thrombosis has been overwhelming to the extent that homocysteine level in blood is now measured and classified,[11] much as blood cholesterol level is.

Blood samples for serum homocysteine estimation are drawn after a 12-hour fast, with levels between 5 and 15 μmol/L regarded as normal. Abnormal concentrations are classified as moderate (16–30 μmol/L), intermediate (31–100 μmol/L) and severe (>100 μmol/L).[11] However, theories on the mechanisms by which homocysteine triggers thrombosis are sparse.[12-14]

Homocysteine biochemistry and metabolic pathways

The four-carbon backbone of homocysteine enables it to form homocysteine thiolactone, an internal cyclic anhydride with a five-member ring. This forms stable salts with strong acids, but when neutralised with weak bases the ring opens and forms two peptide bonds between two molecules of thiolactone, producing homocysteine diketopiperazine. Hydrolysis of homocysteine thiolactone with strong bases also produces homocysteine (Fig. 1).

Homocysteine thiolactone can also convert low-density lipoprotein (LDL) to small, dense particles that are associated with an increased risk of vascular disease.[15] Homocysteine becomes linked to the apolipoprotein B of LDL by peptide-bound homocysteinyl groups, resulting in aggregation and precipitation of the LDL particles. Homocysteine-LDL aggregates are taken up by macrophages to form foam cells, which, in the artery wall,

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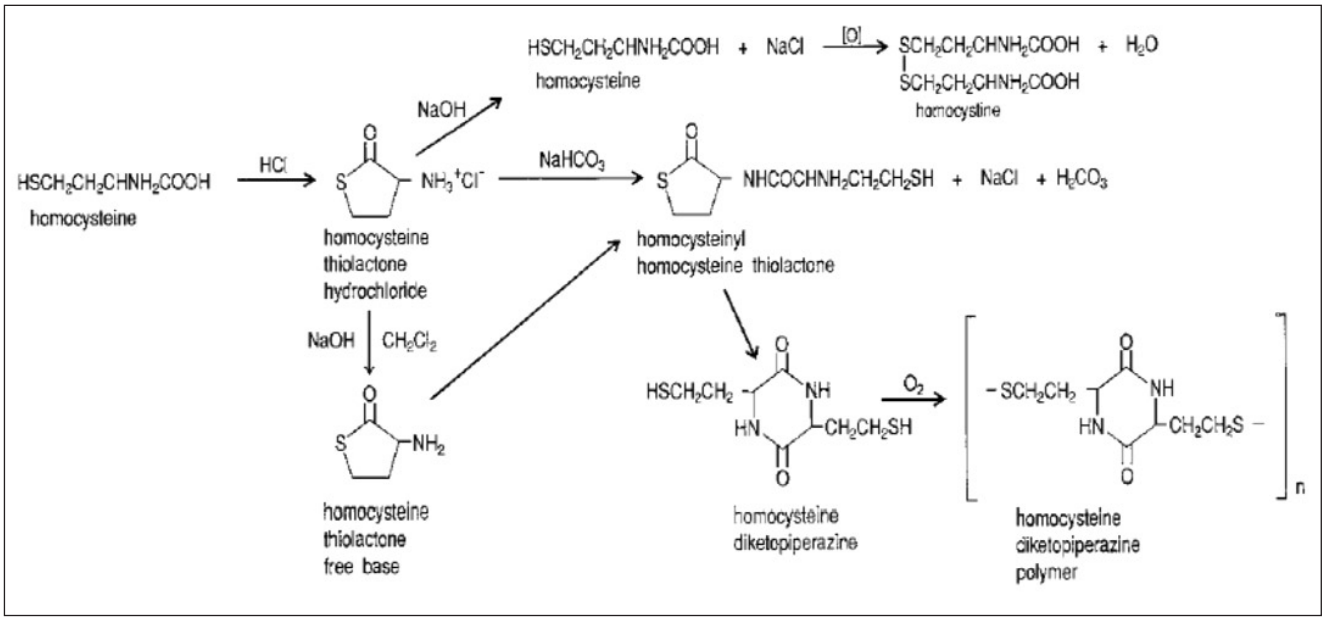

Fig. 1. Reactions involving homocysteine and homocysteine thiolactone, as described in reference 5.

lead to deposition of cholesterol and fats within arteriosclerotic plaques induced by the effect of homocysteine on arterial cells. These observations show that LDL is a carrier of homocysteine in the pathogenesis of arteriosclerotic plaques.

Large amounts of homocysteine have been known to increase the production of cholesterol, triglycerides and LDL. The thioretinaco ozonide theory of homocysteine participation in oxidative metabolism offers a biochemical explanation for this effect.[16] Briefly, inhibition of oxidative metabolism by hyperhomocysteinaemia is caused by depletion of thioretinaco ozonide. Theoretically, hyperhomocysteinaemia causes a depletion of thioretinaco ozonide because an increase in homocysteine thiolactone can displace thioretinamide from thioretinaco ozonide to form a complex compound, thioco, from homocysteine thiolactone and cobalamin.[16] Owing to the decreased oxidative metabolism resulting from depletion of thioretinaco ozonide, excess acetyl coenzyme-A accumulates within mitochondria and is converted to fatty acids and cholesterol.

Adenosyl methionine also plays an important role in homocysteine biochemical pathways. Abnormal elevation of plasma methionine may occur as a result of several different genetic abnormalities, including deficiency of cystathionine-β-synthase (CBS) or of methionine adenosyltransferase (MAT) I and III isoenzymes.[17] An interesting case of fatal homocystinuria in a two-month-old child at the Massachusetts General Hospital in the USA illustrates this point.[5] The baby was found to have both homocysteine and cystathionine in the urine, indicating that a peculiar enzyme deficiency caused his metabolic abnormalities. Enzyme analyses revealed that a methionine synthase deficiency was responsible for homocysteine and cystathionine excretion. This enzyme is dependent upon methyltetrahydrofolate and methylcobalamin for its ability to convert homocysteine to methionine. Biochemical pathways involved in this scenario are shown in Figure 2.

Basically, homocysteine is a thiol-containing amino acid that is metabolised via different pathways. Enzymes such as cystathionine synthase and methylenetetrahydrofolate reductase, together with several co-factors such as vitamin B_6 and folate, are required for this metabolism. In addition, abnormal methylation processes can have toxic effects.[18]

Pathophysiology

The oxidative damage caused by hydrogen peroxide production during the metal-catalysed oxidation of homocysteine is thought to be one of the major pathophysiological mechanisms that lead to thrombosis. Zappacosta *et al*.[19] employed a very sensitive and accurate method to measure the effective production of hydrogen peroxide during homocysteine oxidation. They also investigated the interaction of homocysteine with powerful oxidising species such as hypochlorite, peroxynitrite and ferrylmyoglobin, in order to ascertain the putative pro-oxidant role of homocysteine.

Results showed that only a very small amount of hydrogen peroxide was produced per mole of homocysteine. Moreover, homocysteine strongly inhibited the oxidation of luminol and dihydrorhodamine by hypochlorite or peroxynitrite and rapidly reduced ferrylmyoglobin, the oxidising species, to metmyoglobin. Thus, the commonly held view that homocysteine oxidation is one of the main causative mechanisms of cardiovascular damage has been disputed.

Auto-oxidation of homocysteine to homocystine could result in cellular toxicity, and subsequent endothelial damage occurs through a mechanism similar to that of cystine precipitation, which is well known to cause stone formation in cystinuria.[20] Only traces of homocysteine circulate in plasma as the free thiol; the remainder is present as oxidation products. Of these, the symmetric disulphide homocystine is virtually insoluble at neutral pH and its saturation limit is approximately equal to the concentration of homocysteine in normal plasma. Therefore, a transient increase in homocysteine level could lead to precipitation of homocystine microcrystals in the blood, resulting in damage to the vascular lining.

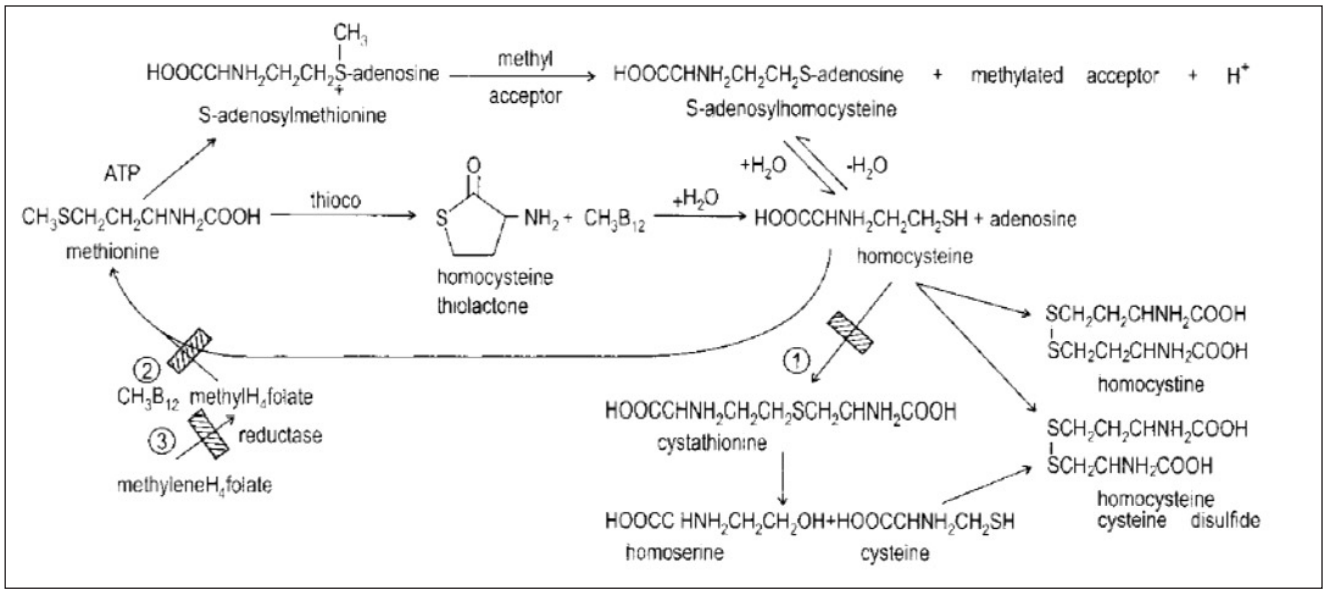

Fig. 2. Illustration by McCully[3] of how inherited deficiencies of the enzymes cystathionine synthase, methionine synthase and methylenetetrahydrofolate reductase (denoted as 1, 2 and 3, respectively) result in accumulation of homocystine and homocysteine cysteine disulphide in tissue and body fluids.

The endothelium exerts fundamental control over vascular tone, coagulation and fibrinolysis. Acute and chronic exposure to homocysteine induces impairment of endothelial function and can give rise to altered haemostasis and morphological changes in the vessel wall. Investigations into the role of homocysteine in endothelium-dependent function in both healthy subjects and cardiovascular patients have been carried out recently[21] and have added important clinical insight into the treatment of cardiovascular disease.

The damaging effects of hyperhomocysteinaemia on endothelial function are partly reversible in patients with established vascular disease, supporting the hypothesis that homocysteine reduction through vitamin supplementation may have vasoprotective effects.[21] In a recent study, 15 patients scheduled for elective percutaneous transluminal coronary angioplasty (PTCA) with plasma homocysteine levels ≥ 16 μmol/L were randomised for six months of treatment with 5 mg folic acid and 0.4 mg cobalamin daily or with a placebo.[22] Coronary endothelial function was evaluated in a non-PTCA vessel using well-regulated acetylcholine infusion and endothelium-dependent volumetric coronary blood flow (CBF) was determined using intracoronary Doppler velocity and quantitative coronary angiography at baseline and after six months. In the folic acid/cobalamin-treated group, CBF increased by 96% after acetylcholine infusion. In contrast, CBF decreased by 16% in the placebo group. These results suggest that coronary endothelial function improves after treatment with folic acid and cobalamin.

Li *et al*.[23] tested the hypothesis that homocysteine reduces intracellular nitric oxide (NO) concentrations and stimulates superoxide (O^{-2} radical) production in the renal arterial endothelium, resulting in endothelial dysfunction. Using fluorescence microscopic image analysis, a calcium ionophore and bradykinin were found to increase endothelial NO in freshly dissected lumen-opened small renal arteries loaded with 4,5 diaminofluorescein diacetate. Preincubation of the arteries with L-homocysteine significantly attenuated the increase in endothelial NO. However, L-homocysteine had no effect on NO synthase activity in the renal arteries but it reduced endothelial NO by scavenging action.

As other thiol compounds such as L-cysteine and glutathione were also found to reduce NO, it is possible that decreased NO is not the only mechanism that results in endothelial dysfunction or arteriosclerosis in hyperhomocysteinaemia. On analysis of intracellular superoxide levels using dihydroethidium trapping, among the thiol compounds studied only L-homocysteine markedly increased superoxide levels in the renal endothelium.[23] These findings indicate that L-homocysteine inhibits the agonist-induced NO increase but stimulates superoxide production within endothelial cells, and may contribute to endothelial injury associated with homocysteine.

Recently, a study was undertaken to determine the effect of homocysteine on the L-arginine/NO pathway in human platelets. [24] In the procedure adopted, human platelets were washed and then incubated with and without homocysteine for 2 h at 37°C, followed by measurement of indices associated with the L-arginine/NO pathway. Homocysteine produced a concentration-dependent reduction in the platelet uptake of L-[H-3]arginine and also a concentration-dependent decrease in nitrite production concurrent with a decrease in cyclic guanosine monophosphate. However, NO synthase activity in platelets, measured as the conversion of L-[H-3]arginine to L-[H-3]citrulline, remained unchanged on incubation with homocysteine. These observations suggest that the L-arginine/NO pathway is involved in the mechanism responsible for homocysteine's effect on platelets by diminishing NO production through decreased uptake of L-arginine.

There is evidence to suggest that high homocysteine levels can cause increased platelet activity in blood, thus increasing the risk of thrombosis.[25] Oxygen-free species produced by homocysteine metabolism and auto-oxidation might be important here. Signorello *et al*.[26] studied the effect of homocysteine on arachidonic acid release in human

platelets. Two important products of arachidonic acid metabolism, thromboxane B-2 (TXB2) and ROS, were assayed. Results indicated that homocysteine induces arachidonic acid release that is partially inhibited by 5,8,11,14-eicosatetraynoic acid (ETYA). Platelet incubation with homocysteine significantly increases basal levels of TXB2 and ROS and this effect is time- and dose-dependent.

In this study, TXB2 formation correlated with arachidonic acid release, and ROS accumulation was largely inhibited by ETYA and partially reduced by diphenyleneiodonium (DPI), suggesting that both enzymes were involved in metabolising arachidonic acid. Thus, it can be concluded that homocysteine induces oxidative stress in human platelets. The imbalance in the platelet redox state and the increased TXB2 formation may result in hyperactivation, contributing to a thrombogenic state that leads to cardiovascular disease.

Epidemiology of homocysteine-related thrombosis

Hereditary issues and ethnic origin

The 677C → T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene is an important cause of mild hyperhomocysteinaemia, but this polymorphism does not appear to be a risk factor for venous thrombosis,[14] although it can cause renal artery thrombosis.[27]

Plasma homocysteine exists in reduced, free oxidised and protein-bound forms. Morelli *et al*.[28] measured the fasting total homocysteine (tHcy) and the MTHFR C677T mutation in 91 patients with venous thromboembolism and without acquired thrombophilia, and in 91 age- and sex-matched controls. Hyperhomocysteinaemia was detected in 11 patients (12.1%) and in two controls (2.2%), resulting in an odds ratio (OR) of 6.1 for venous thrombosis. After excluding 21 patients and four controls with other known genetic risk factors for venous thrombosis, the OR changed only to 7. The prevalence of the MTHFR 677TT genotype was not significantly different between the patient (9.9%) and control (5.5%) groups, with an OR of 1.8 for venous thrombosis.

Subjects with the MTHFR 677TT genotype showed higher tHcy levels than those with the 677CC genotype did, in both the patient and the control groups. Thus, it can be concluded that hyperhomocysteinaemia is a risk factor for venous thrombosis in patients without known acquired thrombophilia and other genetic risk factors for venous thrombosis. Although tHcy levels are significantly higher in those homozygous for the MTHFR C677T mutation, this genotype does not increase the thrombotic risk.

Martinelli *et al*.[29] studied 169 relatives of patients diagnosed with hyperhomocysteinaemia after they developed arterial or venous thrombosis. Approximately 17% had hyperhomocysteinaemia and the relative risk of thrombosis in this group, compared to those without hyperhomocysteinaemia, increased by 20%. They concluded that the low prevalence of hyperhomocysteinaemia among relatives of patients with this metabolic disorder, and their low risk of thrombosis, does not justify family screening.

Homocysteine level shows significant dependence on ethnic origin. In Taiwanese Chinese, hyperhomocysteinaemia is not a risk factor for venous thromboembolism.[30] Tan *et al*.[31] concluded that Asians possess higher levels of homocysteine, a view confirmed in another study carried out in south London by Cappuccio *et al*.[32] This population-based cross-sectional study looked at tHcy levels in different ethnic groups. Fasting plasma tHcy was measured in 1392 men and women (age range 40–59 years old; Caucasian: 475; African: 465 [West African: 180, Caribbean: 280] and South Asian: 452 [Hindus: 222, Muslims: 167). Total Hcy levels were significantly higher in Hindus than in the Caucasian group. In contrast, Muslims had similar tHcy levels to Caucasians, while both West Africans and Caribbeans had slightly lower levels, although the differences were not significant. These differences, presented after adjustments for age, sex, smoking, body mass index and socio-economic status, are large enough to be important contributors to the risk of vascular disease and may be preventable by simple targeted population strategies. However, the higher levels of tHcy among Hindus may in part be due to their vegetarian lifestyle and is discussed later.

Homocysteine level in black people is generally lower than in Caucasians in the same population and this is thought to be due to a different metabolism route for plasma homocysteine. In a study by Simpore *et al*.,[33] healthy black adults who were lifelong inhabitants of Burkina Faso were compared with healthy Caucasians born in Italy but who had lived in Burkina Faso for at least five years. Controlled diets were assigned to all subjects for two weeks before the study. After an overnight 12-hour fast, a methionine-loading test was performed in all subjects. Plasma levels of tHcy, cysteine, glutathione, and cysteinylglycine were measured simultaneously using high-performance liquid chromatography after fasting (baseline) and at either four and eight hours or at two, four, six and eight hours after methionine loading. During the 12 hours after loading, the subjects were monitored and results were analysed using Student's *t*-test and Mann-Whitney U test.

A combination of 17 black adults (9 males, 8 females; median age: 21 years) and 17 Caucasian adults (8 males, 9 females; median age: 35 years) were investigated. Mean plasma tHcy, cysteine, and glutathione levels increased from mean baseline levels more slowly in the black group and peaked at eight hours after methionine loading. In the Caucasian group, these levels peaked four hours after loading. Only mean plasma cysteinylglycine level decreased significantly in the black group after four hours, followed by an increase after eight hours. In the Caucasian group, a less remarkable change in mean cysteinylglycine level was observed, with a peak after four hours.

The researchers concluded that, in addition to lower plasma tHcy levels, the metabolism of plasma tHcy is different in black people than in Caucasians after methionine loading. This difference may be due to different diets and reduced availability of methionine. Moreover, the higher plasma levels of glutathione before and after methionine loading appear to occur exclusively in black people and correspond with variations in cysteinylglycine, suggesting that, in addition to nutritional factors, a racial component may contribute to the difference in plasma tHcy levels. This also might (in part) explain the lower prevalence of coronary heart disease in black people living in Burkina Faso compared with that in other populations.

Gender and age

Lin *et al*.[30] studied a single ethnic group and concluded that sex and age does affect plasma homocysteine, with the level in men up to 25% higher.[32] In women, homocysteine level can decrease during pregnancy and this reduction is not totally explained by the possible extra intake of folates.[34] According to Tan *et al*.,[31] hyperhomocysteinaemia is an independent risk factor for ischaemic stroke in young Asian adults. The relationship between increasing homocysteine and stroke risk is strong, graded and significant.

The association of homocysteine with large-artery strokes suggests that hyperhomocysteinaemia may increase stroke risk via a proatherogenic effect. In their study, Tan *et al*. considered the data from 109 consecutive young hospitalised ischaemic stroke patients (aged <50 years) and 88 age/gender-matched hospital-based controls over a period of 18 months. Fasting homocysteine, vitamin B_{12} and folate were assayed. Mean fasting homocysteine levels were significantly higher in cases than in controls. Mean vitamin B_{12} levels were significantly lower in cases than in controls but folate levels were not significantly different. Mean homocysteine levels were significantly elevated in large-artery strokes. Compared with the lowest homocysteine quartile, the highest quartile was significantly associated with an adjusted OR of 4.3 for ischaemic stroke and 25.3 for large-artery stroke. Using a logistic regression model, the adjusted OR was 5.17 for every 1 μmol/L increase in (log) homocysteine.

In the Hordaland homocysteine study,[35] a comparative analysis of homocysteine and its effect on cardiovascular disease (CVD) in two age groups (40–42 and 65–67) in the western region of Norway over a six-year period was performed on data from 17,361 subjects. At baseline, participants with pre-existing CVD had higher mean tHcy values than individuals without CVD. Only in the older age group, however, did risk of CVD hospitalisation increase significantly with increasing baseline tHcy. The relationship between tHcy level and CVD hospitalisation was significantly stronger among individuals with pre-existing CVD than those without. The findings of this study are compatible with the theory that tHcy interacts with conventional CVD risk factors to provoke acute CVD.

Plasma homocysteine levels also influence the age of onset of thrombosis[36] and paediatric protocols now demand homocysteine level measurements if children show acquired or inherited prothrombotic risk factors.[37] Although there is considerable epidemiological evidence to indicate a relationship between plasma homocysteine level and cardiovascular disease, not all prospective studies support such a relationship. Furthermore, data concerning the role of hyperhomocysteinaemia in patients with premature coronary artery disease (CAD) are rare.

Nikfardjam *et al*.[38] investigated a possible association in young patients between plasma homocysteine level and the extent of CAD and history of myocardial infarction (MI). A cohort of 94 patients was examined for conventional risk factors and a history of previous transmural MI. Coronary angiography was performed to assess the anatomical extent of vessel disease. Plasma homocysteine levels were measured using a commercial enzyme-linked immunosorbent assay (ELISA). It was found that only a history of previous MI was significantly associated with hyperhomocysteinaemia. There was no relationship between elevated homocysteine levels and the anatomical extent of vessel disease in patients with premature CAD.

These results suggest that hyperhomocysteinaemia is an independent risk factor for acute coronary thrombosis rather than for the development of coronary sclerosis. Thus, hyperhomocysteinaemia may influence the clinical situation after plaque rupture not only by prothrombotic action but also by favouring endothelial dysfunction and vasospasm.

Diet, vitamin intake and other factors

Vegetarians can have a plasma homocysteine level 25% higher than those who consume other diets; [32] however, dietary restrictions that impair the intake of folates and vitamin B can be the cause of this.[39] A recent study that followed 80,000 women for 14 years found that the incidence of heart attacks was lowest among those who used multivitamins or had the highest intake of folic acid and vitamin B_6 from dietary sources.[40] These data parallel the current commonly reported finding that elevated homocysteine levels are associated with a higher incidence of heart disease, but in the majority of studies only folic acid (and not homocysteine or vitamin B_{12}) was measured. Also, a common assumption was that low folic acid level was due to inadequate dietary intake.

Zhou *et al*.[41] showed that diet-induced hyperhomocysteinaemia promotes early atherosclerosis and plaque fibrosis but does not, even in the long term, weaken collagen or induce plaque rupture. They came to this conclusion by evaluating the short- and long-term effects of hyperhomocysteinaemia on plaque size and structure in 99 atherosclerosis-prone apolipoprotein E-deficient mice. Hyperhomocysteinaemia was induced by methionine or homocysteine supplementation, both of which raised plasma tHcy levels four to 16 times those observed in mice fed a control diet.

Compared with the control group, aortic root plaque size was significantly larger in supplemented groups after three months but not after 12 months. Hyperhomocysteinaemia was associated with an increase in the amount of collagen in plaques after both three and 12 months. Mechanical testing of the tail tendons revealed no weakening of collagen after hyperhomocysteinaemia for 12 months. Many plaques in both the control and supplemented groups appeared rupture prone morphologically, but all aortic root plaques and all but one coronary plaques had an intact surface without rupture or thrombosis.

Homocysteine can stimulate procoagulant factors and/or impair anticoagulant mechanisms or fibrinolysis. Klerk *et al*.[42] performed an intervention study and examined the effect of homocysteine lowering by B-vitamin supplementation on prothrombin fragments, thrombin-antithrombin complex (TAT) and fibrin degradation products (D-dimer). The study looked at 118 healthy volunteers, 50 with homocysteine >16 μmol/L and 68 with homocysteine ≤16 μmol/L. The subjects were randomised to either placebo or high-dose B-vitamin supplements (5 mg folic acid, 0.4 mg hydroxycobalamin, and 50 mg pyridoxine) daily for eight weeks. Although homocysteine concentrations were reduced by 27.7% in the B-vitamin group compared with the placebo group, no effect on prothrombin fragment or TAT concentrations was observed. A 10.4% reduction was observed for D-dimer. These results suggest that homocysteine reduction by B-vitamin supplementation in healthy subjects has a modest beneficial effect on clotting activation.

The American Heart Foundation information service has identified several cases in which people suffering from coronary artery disease had fatal levels of homocysteine despite taking higher than the recommended dose of vitamin supplements. One case involved a 60-year-old man who had undergone bypass surgery but who repeatedly suffered angina and showed significant re-occlusion of the coronary vessels verified by angiography. This patient knew about the dangers of homocysteine and had been taking more than 15 mg folic acid a day, along with other homocysteine-lowering vitamins. Blood test showed a very high homocysteine reading of 18 μmol/L; however, immediate daily intake of 6 g trimethylglycine (TMG) resulted in a reduction of this level to 4 μmol/L within a month.

Another case involved a healthy person who took a daily supplementation of 0.5 g TMG, 4 mg folic acid, and high doses of many other vitamins. Blood test revealed a homocysteine level of 11.3 μmol/L, which is far above the safe range of <7 μmol/L. Subsequently, 6 g TMG and 500 mg vitamin B_6 were added to his daily intake, and homocysteine level dropped to <6 μmol/L within 60 days.

In response to these cases, the American Heart Foundation analysed all the homocysteine tests it had conducted and discovered that 62% of members tested had excess homocysteine in their blood (Table 1). The most recent survey[44] showed that the average homocysteine level of an American has been reduced to 10 μmol/L, thus dietary supplements have proved effective in suppressing dangerously high homocysteine levels.[45]

Several drugs have been shown to affect homocysteine levels.[46] Some drugs frequently used in patients at risk of cardiovascular disease, such as the fibric acid derivatives used in certain dyslipidaemias and metformin in type II diabetes mellitus, also raise plasma homocysteine levels. This elevation poses a theoretical risk of negating some of the benefits of these drugs that alter plasma homocysteine levels by different mechanisms. For example, drugs such as cholestyramine and metformin interfere with vitamin absorption. Interference with folate and homocysteine metabolism by methotrexate, nicotinic acid and fibric acid derivatives can also lead to increased plasma homocysteine levels.

A major unanswered question is whether homocysteine is causally involved in disease pathogenesis or is simply a passive and indirect indicator of a more complex mechanism.[47] S-adenosylmethionine and S-adenosyl-homocysteine (SAH) are important metabolic indicators of the cellular methylation status. The former is the substrate and the latter is the product of methyl-transferase reactions. Chronic elevation in homocysteine level results in parallel increases in intracellular SAH and potent product inhibition of DNA methyltransferases. SAH-mediated DNA hypomethylation and associated alterations in gene expression and chromatin structure may provide new hypotheses for pathogenesis of diseases involving homocysteinaemia.[47,48]

Legendre *et al*.[49] suggested that it is uncertain whether increases in plasma homocysteine represent a cause or a consequence of the disease process, and they studied the effect of fenofibrate on tHcy in PPARalpha-deficient mice, as well as the effect of fenofibrate (with and without folate) supplementation on total and protein-bound homocysteine in rats. Fenofibrate significantly increased serum tHcy in wild-type mice but not in PPARalpha-deficient mice. In rats, fenofibrate increased serum tHcy by 69% (only the protein-bound fraction was increased) while the co-administration of fenofibrate and folate increased tHcy by only 7%. Fenofibrate also induced a significant increase in tHcy.

Table 1. Summary of results of American Heart Foundation analysis of homocysteine determinations.[43]

Risk	Serum homocysteine (μmol/L)	%
Low	0–6.3	38
Moderate	6.3–10	52
Highest	>10	10

As yet there is no correlation of this finding to human subjects, but these results suggest that the extent and mechanism of the increase in tHcy in patients treated with fenofibrate should not necessarily be associated with relevant risk.

Homocysteine measurement

Publication of risk bands (Table 1) that relate homocysteine and thrombosis puts the spotlight on the reliability of homocysteine determination, of which there are several methods available currently. A comprehensive review of the performance of the different homocysteine assays, including sample volume, analytical imprecision, throughput and quality control schemes has been published recently.[50]

Ducros *et al*.[51] evaluated the interchangeability of tHcy measurements in nine French hospital laboratories. Six methods were used and included gas chromatography–mass spectrometry (GC–MS), high-performance liquid chromatography (HPLC) with fluorescence detection, fluorescence polarisation immunoassays (FPIA), enzyme immunoassay (EIA), amino acid analysis and capillary electrophoresis coupled with laser-induced fluorescence detection (EC-LIF). Each laboratory analysed samples from 41 patients in which eight samples contained additional homocysteine. Results were analysed for imprecision, recovery and methodological differences.

Mean among-laboratory imprecision ranged from 13 to 18% and was identical to the mean among-method variation. Immunoassays tended to produce underestimates. The bias relative to the GC-MS method was less than ±12.5%, except in results produced by two laboratories (one used FPIA and the other EC-LIF). Thus, between-laboratory variability in tHcy results was deemed unsatisfactory and does not permit the evaluation of cardiovascular risk linked to moderate increases in tHcy. However, as shown in the recent review by Clarke and Stansbie,[52] results of a multicentre European demonstration project[53] showed that homocysteine immunoassay could be used safely in place of HPLC or GC-MS.

In summary

Over the past few years, elevated blood homocysteine levels have been linked to an increased risk of thrombosis, even

in people with normal cholesterol levels. Intervention trials (e.g., with vitamins) to reduce homocysteine suggest that this does lead to improvements in endothelial, platelet and coagulation markers. As yet, however, no studies point to an effect in reducing end-points, and large, well-controlled trials will be needed to determine this in the future. □

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