Homocysteine and coronary artery disease

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■ **BACKGROUND** Homocystinuria is a rare autosomal recessive disease complicated by early and aggressive occlusive arterial disease. This may be related to the grossly increased homocysteine concentrations seen in this disease. More recently, milder hyperhomocysteinemia has been proposed as an independent risk factor for coronary artery disease.

■ **SUMMARY** Many patients with homozygous homocystinuria develop severe premature atherosclerosis and thromboembolism, probably caused by abnormally high concentrations of homocysteine. Homocysteine undergoes metabolism either by remethylation or transsulfuration, and deficiency or dysfunction of any of the substances that regulate these reactions may lead to hyperhomocysteinemia. Homocysteine may have adverse effects on platelets, clotting factors, and endothelial cells. Studies have demonstrated significantly higher plasma homocysteine levels in patients with occlusive arterial disease than in controls. The causes are not clearly understood but may include deficiency of vitamin B6, vitamin B12, and folic acid and heterozygosity for cystathionine synthase deficiency. Vitamin supplementation can lower plasma homocysteine levels.

■ **CONCLUSIONS** Whether measuring plasma homocysteine levels in patients with coronary artery disease should be routine and whether treating hyperhomocysteinemia in these patients may reduce the risk of coronary events remains to be determined.

■ INDEX TERMS: HOMOCYSTEINE; HOMOCYSTINURIA; CORONARY DISEASE; VASCULAR DISEASES ■ CLEVE CLIN J MED 1994; 61:438–450

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HOMOCYSTINURIA might have remained an obscure hereditary metabolic anomaly were it not that afflicted patients suffer early, severe arterial disease, possibly due to the extremely high plasma homocysteine concentrations seen in this disorder. Of greater interest, patients with coronary artery disease have higher plasma concentrations of homocysteine than do normal controls, even among people who do not have the genetic trait for homocystinuria. Further, relatively benign interventions—supplements of vitamins B6 and B12 and folic acid—may decrease plasma homocysteine concentrations.

These intriguing observations have led researchers and clinicians to wonder whether measuring homocysteine concentrations might identify people at risk for coronary artery disease, and whether lowering homocysteine concentrations might decrease the risk. Many questions remain to be answered, however, before such an approach can be advocated.

WHAT IS HOMOCYSTINURIA?

Homocystinuria, a rare inborn error of metabolism, is an autosomal recessive disease; the usual underlying genetic abnormality is a deficiency of cystathionine synthase. The condition was first described in Ireland in 1962 by Carson and Neill[1] after a survey of mentally handicapped children. A further description by Gerritsen[2] followed almost immediately. Two years later, Mudd and others demonstrated the responsible enzyme defect in a liver biopsy specimen from a homocystinuric patient.[3]

Superficially at least, the disorder is not unlike Marfan's syndrome: tall stature and abnormalities of the bones and eyes are common. Mental retardation is an important distinguishing sign in homocystinuria, but for those interested in vascular disease, the more striking features are the episodes of arterial occlusion and venous thromboembolism that may appear at an early age, affect any or all parts of the arterial tree, and are the principal cause of death in these patients. These complications are common, and approximately 25% of people with homocystinuria have a thromboembolic event by the age of 20.[4] This aspect of homocystinuria has recently received closer scrutiny and has led to extensive work on the epidemiologic, clinical, and pathophysiologic links between homocysteine and vascular disease.

In the original case reports it was natural to refer to the presence of homocystine in the urine as "homocystinuria." But as further clinical observations were made and the gamut of signs was recognized, it quickly became apparent that this is only one abnormality in a wider syndrome, and homocystinuria hence acquired the additional, broader meaning of the disorder itself.

Although the presence of any homocystine in the urine is usually pathologic, low concentrations of thiols (including homocysteine and related compounds) in the plasma are normal. A plasma homocysteine concentration greater than 15 μmol/L is now termed "hyperhomocysteinemia." While exceptionally high levels may occur in the syndrome of homocystinuria, milder degrees of elevation may be seen in a variety of other conditions, which will be discussed later.

VASCULAR PATHOLOGY

Following the discovery of homocystinuria, the complications of severe premature arteriosclerosis and thromboembolism became evident.[3,4] Large, medium-sized, and small arteries in any vascular bed may be affected: the intima thickens, medial muscular fibers split and fray, and the internal elastic membrane undergoes similar changes. The lesions, which may be focal, are associated with proliferation of perivascular connective tissues containing increased numbers of fibroblasts, collagen bundles, and small elastic fibers. Fibrosis and thickening of the intima and the media occurs and may in severe cases lead to intraluminal narrowing and to arterial obstruction.[3,5] Similar venous lesions are rare, although both arterial and venous thromboemboli are common. Venous thromboembolism accounts for over 50% of all vascular events in patients with homocystinuria.[4]

PATHOPHYSIOLOGY

Considerable evidence suggests that these atherogenic and thrombotic tendencies are caused by abnormally high concentrations of homocysteine. For example, similar vascular lesions have been reported in patients with inherited remethylation enzyme defects, which are also characterized by gross hyperhomocysteinemia and excretion of homocystine in the urine. In these disorders, the high methionine concentration that is seen in classic homocystinuria due to cystathionine synthase deficiency is absent. This suggests a role for homocysteine or its derivatives, but not for methionine, in the development of vascular disease.[4,5]

Endothelial damage

Evidence that homocysteine has a direct toxic effect on endothelial cells has been derived both from in vitro studies in human cell cultures[6] and from in vivo animal models (baboons).[7] Short-term intravenous infusion of homocystine results in desquamation of endothelial cells and has been associated with arterial damage similar to early human atherosclerosis. In pigs fed a diet deficient in pyridoxine (resulting in vitamin B6 deficiency), a rise in homocysteine concentration was associated with focal vascular damage. Indeed, widespread vascular changes were noted as long ago as 1949 in monkeys with induced vitamin B6 deficiency.[8] Not all these findings have been consistent, however, and other studies in rabbits, monkeys, and pigs have failed to show similar vascular changes related either to infusions of homocysteine or to diets deficient in pyridoxine.[9,10] These variable findings may

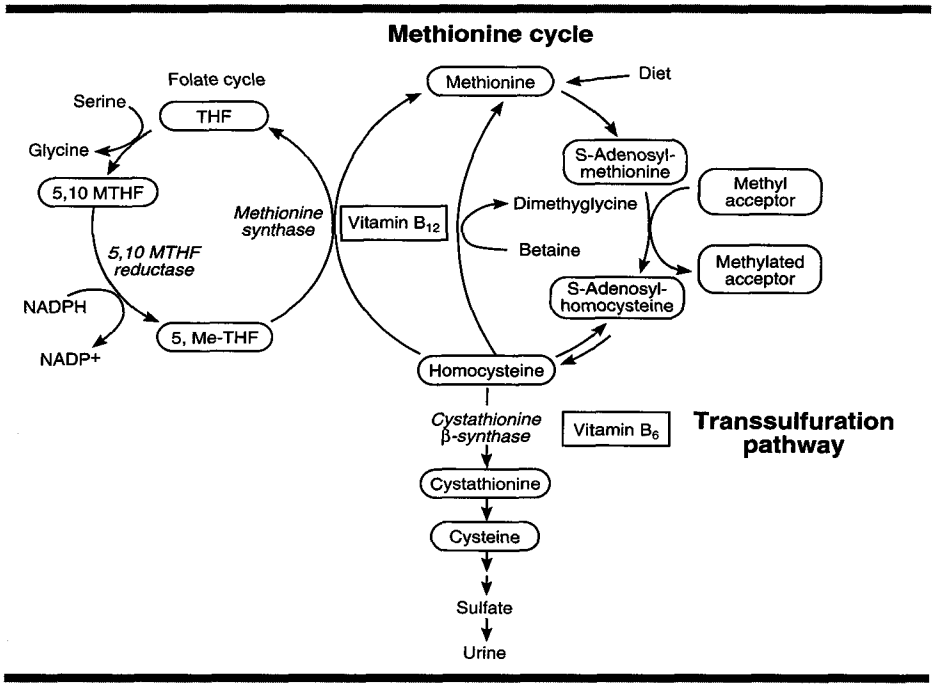

FIGURE 1. The metabolic pathways for the metabolism of homocysteine. THF, tetrahydrofolate; MTHF, methylenetetrahydrofolate; Me-THF, methyltetrahydrofolate.

reflect differences in the substances infused, the duration of infusion, the laboratory models, and the naturally occurring diseases. In addition, homocysteine thiolactone was used in some of the studies and may be independently cytotoxic.[11] Further complicating the matter, many of the studies used D,L-homocysteine, which contains an unnatural D isomer with unknown effects. Species-dependent responses may also play a role.

The mechanism of homocysteine-associated endothelial damage remains unclear, but it may be inhibited by the addition of catalase, suggesting that hydrogen peroxide produced during oxidation of homocysteine plays a role.[6] This may require the participation of other agents such as iron or copper; at least in patients with homocystinuria, increases in circulating copper concentrations have been observed.[10] Recent studies of bovine endothelium have suggested that endothelium-derived relaxing factor may protect against this adverse effect of homocysteine,[11] but this protection may be lost with progressive endothelial damage.

Researchers have used flow-mediated dilation to demonstrate endothelial dysfunction in vivo in humans with homozygous homocystinuria.[12] This may be evident as early as 4 years of age. Such abnormalities were not seen in their heterozygous parents, however, and the technique has not yet been applied to patients with vascular disease.

Coagulation disorders

A number of studies have pointed to possible abnormalities of platelet function. For example, L-homocystine or D,L-homocysteine added to platelets in vitro may increase synthesis of thromboxane B2 and production of 12-hydroxy-5,8,10-heptadecatrienoic acid.[13] Increased adhesiveness, abnormalities of interaction with adenosine diphosphate (ADP) and of ADP-induced platelet aggregation, and impaired platelet survival have all been demonstrated, although not consistently.[10] Some of the vascular abnormalities reported in animal studies were prevented by dipyridamole, sudoxicam, or sulfinpyrazone, suggesting that platelets play a role in endothelial damage.[7,14] Recently, elevated thromboxane A2 formation (which may reflect platelet activation) has been reported in patients who have homozygous homocystinuria.[15]

Various abnormalities of clotting have been reported. Reduction of antithrombin III activity to 50% to 75% of normal, a range associated with severe thrombotic tendencies, has been seen in patients with homocystinuria, although not in their heterozygous parents.[16] Homocysteine may cause an endothelial cell factor to activate factor V,[17] although reduced factor VII has also been reported in homocystinuria.[10] In one study, L-homocysteine activated factor XII.[18]

In addition to these effects on clotting, homocysteine may also inactivate endothelial cell anticoagulant protein C. The expression of surface thrombomodulin, which promotes protein C activation and inhibits the procoagulant activities of thrombin, may thus be irreversibly inhibited.[19–21]

Low-density lipoprotein (LDL) cholesterol may become oxidized[22] and may then be metabolized by scavenger-cell surface receptors, potentially causing atherosclerosis. Recent data, however, suggest that elevated plasma homocysteine concentrations do not enhance the oxidative stress of lipid particles.[23] An interaction with lipoprotein(a) that promotes binding with fibrin has, however, been reported and could also be potentially prothrombotic.[24]

In summary, there is conflicting evidence concerning the precise prothrombotic actions of homocysteine on vascular endothelium, platelets, coagulation factors, and lipids. A single identifiable and unifying mechanism that explains the severe and early thromboembolic episodes that complicate homocystinuria has not yet been proposed.

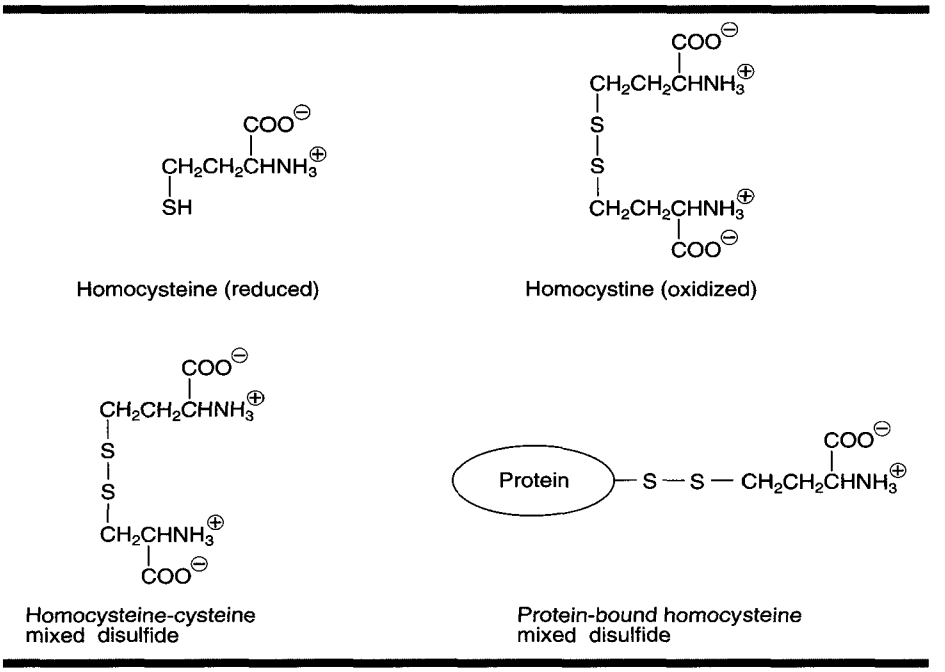

FIGURE 2. The structures of homocysteine and related compounds.

HOMOCYSTEINE METABOLISM AND MEASUREMENT

Familiarity with the two major routes of homocysteine metabolism is important in understanding the biochemical basis for hyperhomocysteinemia and the principles by which the transsulfuration and remethylation pathways can be therapeutically manipulated.

Homocysteine is the demethylated derivative of the essential sulfur-containing amino acid methionine, which is the principal methyl donor in almost all methylation reactions and is derived from dietary protein.[25] After it is formed, homocysteine undergoes either remethylation to methionine or transsulfuration to cystathionine (*Figure 1*). Normally, about 50% of intracellular homocysteine is transsulfurated and 50% is reconverted to methionine by one of two remethylation pathways. In the more important pathway a methyl group is transferred to homocysteine from 5-methyltetrahydrofolate; this reaction is catalyzed by the vitamin B12 (cobalamin)-dependent enzyme 5-methyltetrahydrofolate-homocysteine methyltransferase (methionine synthase). The alternative remethylation reaction, which occurs in the liver and the kidney,[26] involves the irreversible transfer of a methyl group from betaine to homocysteine. Entry of homocysteine into the transsulfuration pathway is catalyzed by vitamin B6 (pyridoxal phosphate)-dependent cystathionine synthase. Homocysteine is first converted to cystathionine, then to cysteine, and finally to sulfate, which is excreted in the urine.

Upon entry into the circulation, up to 90% of homocysteine combines with plasma protein. The remainder auto-oxidizes to form the disulfide dimer homocystine or oxidizes with cysteine to form the homocysteine-cysteine mixed disulfide. The term "homocyst(e)ine" has been used to collectively designate these different species (*Figure 2*). High-performance liquid chromatography can be used to measure the total homocysteine concentration, which usually ranges from 5 to 15 μmol/L.[27,28] A sensitive and reproducible technique for measuring plasma homocysteine has been developed at the Cleveland Clinic that possesses the added advantage of quantifying cysteine and other plasma thiols.[29]

Normal homocysteine metabolism is determined by a number of essential enzymes and vitamins, and the absence or dysfunction of any of these substances

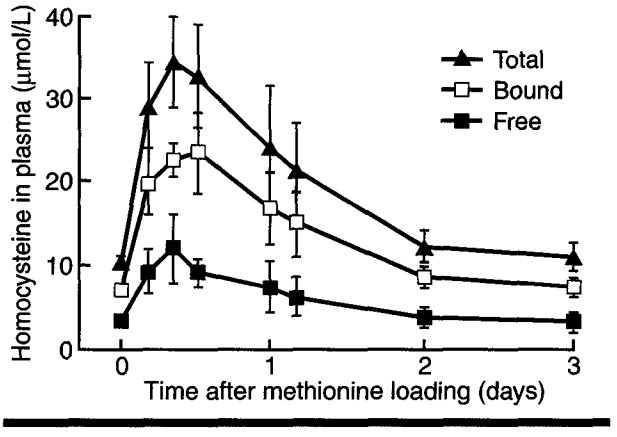

FIGURE 3. The effect of methionine loading on plasma homocysteine concentrations. Although the test is useful as an investigational tool, the measurement of total fasting homocysteine is likely to become the standard test. From Ueland et al, reference 10, courtesy of Marcel Dekker, Inc.

TABLE 1
CAUSES OF HYPERHOMOCYSTEINEMIA

Inherited causes
Transsulfuration abnormalities
Cystathionine synthase deficiency
Remethylation abnormalities
Defective vitamin B12 transport
Defective vitamin B12 coenzyme synthesis
Defective methionine synthase
5,10 methylenetetrahydrofolate reductase deficiency or defects
Acquired causes
Diseases
Chronic renal failure
Acute lymphoblastic leukemia
Psoriasis
Deficiency states
Vitamin B12 deficiency
Folate deficiency
Vitamin B6 deficiency
Drugs
Methotrexate (inhibits dihydrofolate reductase)
Nitrous oxide (inactivates methionine synthase)
6-azauridine triacetate (a vitamin B6 antagonist)
Anticonvulsants, eg, phenytoin and carbamazepine (folate antagonists)

may lead to hyperhomocysteinemia. Gross increases in plasma homocysteine may be seen in homozygous cystathionine synthase deficiency, the most common hereditary cause of homocystinuria. Of equal importance are the enzymes methionine synthase and methylenetetrahydrofolate reductase and their requisite cofactor and cosubstrate, vitamin B12 and methylenetetrahydrofolic acid, respectively.

The methionine-loading test

The plasma homocysteine concentration is usually measured in the fasting state, allowing ready detection of abnormal values. However, some people who have normal fasting levels may nevertheless have a latent potential for developing hyperhomocysteinemia. This condition can be detected in a manner analogous to glucose tolerance testing in diabetes. The patient takes a dose of 0.10 g/kg body weight of methionine by mouth, which normally causes the plasma concentration of homocysteine to rise in a characteristic fashion (*Figure 3*).[10] Recent technical advances in measuring total homocysteine may, however, render this cumbersome and time-consuming test less important in the future.

CAUSES OF HYPERHOMOCYSTEINEMIA

Table 1 outlines the principal causes of hyperhomocysteinemia. Plasma homocysteine concentrations increase with age, and levels are higher in men than in women.[10,29] Data conflict concerning the effect of menopause, but homocysteine concentrations probably do not rise significantly in women at this time.[10,30]

Cystathionine synthase deficiency is the principal cause of the syndrome of homozygous homocystinuria, a rare disease in which total homocysteine levels sometimes reach 500 µmol/L (50 times normal). Cystathionine synthase deficiency alone, however, is not the sole explanation: differences in the physical and immune properties of the enzyme suggest the possibility of mutations of different types.[3] Heterozygosity for cystathionine synthase deficiency may be responsible for moderate elevations in plasma homocysteine levels.[3]

Other well-defined, albeit rare, abnormalities in the remethylation pathway may also cause homocystinuria, including insufficient methionine synthase activity and 5,10-methylenetetrahydrofolate reductase deficiency.[10] Vitamin B12 deficiency also leads to elevated plasma homocysteine concentrations—sometimes even higher than in carriers for cystathionine synthase deficiency.[31] Similarly, acquired deficiency of folic acid due to inadequate intake or to antagonism by drugs may indirectly impair remethylation and result in hyperhomocysteinemia.[32,33] Results conflict concerning the relationship between deficiency of pyridoxal phosphate (the coenzyme for cystathionine synthase) and elevated homocysteine concentrations in humans.[34,35]

HYPERHOMOCYSTEINEMIA AND VASCULAR DISEASE

Soon after the initial reports of homocystinuria from Belfast and Wisconsin, the association of homocystinuria with premature vascular disease became evident. It was only in 1976, however, that a possible link between carriers and premature vascular disease was suggested, when higher mixed homocysteine disulfide levels were observed in 25 Australian patients with coronary artery disease compared with controls.[36]

Some years later, Freedman et al[37] and Murphy-Chutorian et al[38] in the United States also demonstrated possible abnormalities of methionine metabolism in patients with coronary artery disease. The findings were confirmed by Kang et al,[39] who showed in a case-control study that fasting homocysteine levels were higher in patients with coronary artery disease than in controls with normal angiographic findings.

Similar findings have been reported in patients with other premature vascular diseases.[40,41] To date, more than 20 studies involving more than 2000 patients have demonstrated significantly higher plasma homocysteine levels in patients with coronary artery disease, cerebrovascular disease, and peripheral arterial occlusive disease compared with controls.[36–61] The studies have varied markedly in design, types of patients and controls, and definitions of endpoints and of hyperhomocysteinemia. In addition, patients with certain risk factors were included in some studies but were excluded from others, the use of the methionine-loading test varied, and statistical methods differed, resulting in minor but important differences in detail.

What is nonetheless striking is the apparently reliable and reproducible association between high homocysteine levels and vascular diseases in such disparate groups. This constant correlation, despite the different methods used, suggests a true relationship rather than mere coincidence. Indeed, the link may be stronger than currently thought: researchers in Ireland observed unadjusted odds ratios of 40.3 for cerebral vascular disease, 22.3 for peripheral vascular disease, and 23.9 for coronary artery disease in patients with hyperhomocysteinemia compared with controls.[42] Although these ratios were reduced in multivariate analysis, a threefold increase in risk persisted for all forms of vascular disease, and the values for hyperhomocysteinemia were higher than those for hypercholesterolemia, hypertension, and cigarette smoking. The varied populations and methods have hampered formal meta-analysis, but some authors have reported pooled results, with similar conclusions.[10]

Peripheral venous thromboembolism accounts for more than half of the vascular episodes seen in patients with homocystinuria,[3] but higher plasma homocysteine concentrations have not been found in nonhomocystinuric patients suffering venous thromboembolism when compared with controls.[10]

CAUSES OF HYPERHOMOCYSTEINEMIA IN PATIENTS WITH VASCULAR DISEASE

The causes of hyperhomocysteinemia in patients with vascular disease are complex and poorly understood. In normal subjects, age and gender influence homocysteine concentrations. In patients with coronary artery disease, however, age-related changes have been seen in some studies,[39,43,45] but not in others.[38,44] Depletions of cofactors necessary for homocysteine metabolism, or coexisting renal impairment, could account for rising homocysteine concentrations with age. Cystathionine synthase activity may not decline with age in patients with vascular disease, as it does in normal individuals.[62] Small differences between men and women have been seen in one study of patients with coronary artery disease,[39] but not in another,[43] and sex-related differences have not been seen in patients with peripheral vascular[40,46,47] or cerebrovascular disease.[40,48–50]

Deficiency of vitamin B12 and folic acid

Because vitamin deficiency can raise homocysteine concentrations, a number of investigators have measured concentrations of vitamin B12 and folic acid in patients with vascular diseases. Major studies in patients with coronary artery disease have yielded conflicting results, but both folic acid and vitamin B12 concentrations have been reported to be lower in patients with higher homocysteine concentrations.[42,51] In the study of Ubbink et al,[45] although the concentration of folate was low, that of vitamin B12 was normal. Low levels of folic acid or vitamin B12 have also been observed in some studies of patients with peripheral vascular disease[41,42] and stroke,[42,50] but not in others.[52] Similar findings have been seen in other studies of patients with coronary artery disease.[60,61]

TABLE 2
SERUM VITAMIN B12 AND FOLATE CONCENTRATIONS IN PATIENTS WITH AND WITHOUT HYPERHOMOCYSTEINEMIA AT THE CLEVELAND CLINIC FOUNDATION

	Patients with hyperhomocysteinemia	Patients without hyperhomocysteinemia	*P* value
Serum vitamin B12 (pg/mL)	313 ± 136	430 ± 230	.001
Serum folate (ng/mL)	9.8 ± 5.2	11.8 ± 5.5	.07

TABLE 3
PREVALENCE OF HYPERHOMOCYSTEINEMIA AND POSSIBLE HETEROZYGOSITY FOR CYSTATHIONINE SYNTHASE DEFICIENCY IN PATIENTS WITH VASCULAR DISEASE

Country	Reference	Year	Prevalence % Cerebral vascular disease	Peripheral vascular disease	Coronary artery disease
Australia	36	1976	—	—	28
Sweden	54	1984	28	—	—
Netherlands	40	1985	28	28	0
United States	38	1985	—	—	16
Ireland	42	1991	33	22	30

In unpublished work at The Cleveland Clinic Foundation, we found significantly higher mean plasma homocysteine concentrations in patients with coronary artery disease compared with controls, 15.1 vs 10.0 μmol/L. Plasma homocysteine concentrations correlated negatively with both serum vitamin B12 and folate (*Table 2*). Three studies of patients with peripheral vascular disease and stroke yielded similar results.[41,50,52]

Deficiency of vitamin B6

Most studies of coronary artery disease did not include measurements of vitamin B6 concentrations, but these concentrations were normal in two studies.[40,45] A negative correlation between homocysteine and pyridoxal phosphate has recently been reported,[60] and we have found similar results in patients with coronary artery disease. In one study of cerebrovascular and peripheral vascular disease, vitamin B6 concentrations were normal.[40] In two further studies, however, overall pyridoxal phosphate levels were lower in patients than in controls[41] and correlated negatively with plasma homocysteine.[50]

These findings suggest a possible contributory role for vitamin deficiency in the development of vascular disease. The relationship of vitamin deficiency and elevated homocysteine concentrations may provide a rationale for the previously suggested association between pyridoxine deficiency and atherosclerosis,[8] and vitamin deficiency may also be closely linked with enzyme deficiencies that are associated with high homocysteine concentrations.

Heterozygous cystathionine synthase deficiency

Only three clinical studies have formally evaluated cystathionine synthase activity in patients with coronary artery disease.[40,42,58] Presumed carrier rates, estimated from the proportion of coronary patients with hyperhomocysteinemia, have ranged from 0% in Holland to 20% to 30% in Ireland and Australia (*Table 3*).

A further discrepancy exists between the prevalence of heterozygosity for cystathionine synthase deficiency as estimated from the number of heterozygotes among patients with coronary artery disease and the prevalence of heterozygosity based on known cases of homozygous homocystinuria. In Ireland, for example, homocystinuria has a prevalence of approximately 1 in 57 000 births,[3] perhaps the highest in the world. This number of homozygotes implies a carrier frequency of approximately 1% in the general population. In one Irish study, the possible carrier frequency among patients with coronary disease was estimated to be approximately 30%.[42] There are thus too few heterozygotes to account for the numbers seen in the vascular disease population.[63] In the United States, the estimated frequency of homozygous homocystinuria is 1:406 000,[3] yielding a carrier rate of approximately 1 in 300, which is even lower.

This complex issue has been reviewed elsewhere.[63] Heterozygosity for cystathionine synthase deficiency may be infrequent, and acquired and inherited deficiencies of other factors such as cobalamin, folate, or alternative enzymes may be more prevalent than previously thought. The balance between enzyme deficiency on the one hand and vitamin depletion on the other, although of fundamen-

tal importance, remains unclear; there may be a number of abnormalities of variable penetrance of the transsulfuration and remethylation pathways resulting in hyperhomocysteinemia.

OTHER RISK FACTORS

After the association of homocysteine with vascular disease was discovered, its relationship with other risk factors was evaluated. Earlier studies deliberately excluded patients with other risk factors in order to isolate potential confounding variables. However, multivariate analysis has been used in more recent studies. Conflicting results have been reported from different studies on the link between homocysteine and other risk factors, although homocysteine is an independent risk factor in its own right.

In most studies of patients with coronary artery disease[38,44,45,61] or other vascular diseases,[40,46–48,50,54] no apparent relationship between hypercholesterolemia and hyperhomocysteinemia has been seen. Kang et al,[39] however, demonstrated a correlation between plasma homocysteine and cholesterol concentrations in coronary patients, although none were hypercholesterolemic, and Wu et al have recently shown a correlation between homocysteine and LDL cholesterol.[60] Mölgaard et al[52] have found a correlation between total cholesterol and homocysteine in patients with peripheral vascular disease, but not in controls.

Most investigators have been unable to show a link between blood pressure and homocysteine concentrations in patients with coronary artery disease[38,39,44,60,61] or other forms of vascular disease.[47–49,50,52,54] Malinow[46] found that 77% of patients with peripheral vascular disease and elevated homocysteine concentrations were hypertensive, compared with 40% of those with normal homocysteine levels. In patients with vascular disease, smoking did not correlate with homocysteine,[38–40,46–50,52,54,60,61] except in two studies.[55,64] No correlation between diabetes and hyperhomocysteinemia has been found in patients with vascular disease.[38,46,47,49,50,52,60,61]

Although hyperhomocysteinemia appears to be an independent risk factor for vascular disease, its effects could still modify (or be modified by) other risk factors such as smoking, hypertension, or hypercholesterolemia. The possibility of interaction or synergy remains, as interactions between cholesterol and homocysteine have already been noted.[22,60,65]

A large European case-control study focusing on men under age 55 with vascular disease recently evaluated possible relationships between homocysteine and other risk factors.[66] The principal objective was to clarify the relationship between hyperhomocysteinemia and premature vascular disease. Preliminary results suggest that the previous findings from other studies will be corroborated and that higher homocysteine concentrations are indeed independently associated with vascular disease.[59] The work will also yield useful information on possible risk-factor interactions and on the relationship between homocysteine concentrations and vitamin status.

SEVERITY OF VASCULAR DISEASE

Although homocysteine may be a risk factor for vascular disease, its relationship to the severity or extent of underlying arterial abnormalities is unclear. Kang et al[39] reported no correlation between homocysteine concentrations and Gensini scores of severity of coronary disease. However, Ubbink et al[45] showed a positive correlation between homocysteine levels and the number of occluded vessels. In one study, homocysteine concentrations in patients with transient ischemic attacks and those with completed stroke were similar.[49] In an ultrasonographic study, homozygous homocystinuric patients had more severe peripheral vascular lesions than did heterozygotes.[67] Higher homocysteine levels have also been associated with more rapid progression of lower-extremity lesions and with an increased frequency of cardiac events in patients with occlusive peripheral arterial disease.[47] These areas require further study and clarification.

THERAPY OF HOMOCYSTINURIA

A low-methionine diet may lower high plasma homocysteine concentrations in patients with homocystinuria. In addition, such methionine restriction may prevent some of the more serious complications of the disease, such as mental retardation, lens dislocation, and seizures. The effect of diet on other aspects of the disorder such as osteoporosis and thromboembolism are under investigation.

Although diet is the mainstay of treatment, Barber and Spaeth[68] demonstrated that patients could be divided into two broad categories on the basis of their response to vitamin B6. The success of this

treatment in reducing and, in some cases, normalizing plasma homocysteine levels is not due to the correction of an underlying deficiency of vitamin B6, but is attributed to a slight but critical increase in cystathionine synthase activity caused by the doses of vitamin B6 cofactor that are used. The use of folic acid was later proposed when its efficacy in lowering homocysteine concentration by increasing remethylation was noted in a study of patients with homocystinuria undergoing investigation for other reasons.[69]

Reduction of elevated homocysteine concentrations has since been achieved in conditions other than homocystinuria. For example, patients with renal failure[10] and those who have undergone renal transplantation with impaired renal function also develop high homocysteine levels, which may be reduced by the administration of folic acid.[10,70] This effect is evident after 2 weeks of treatment and may be maximal at 4 weeks.[70] In patients with frank folate deficiency, plasma homocysteine is elevated and may also be rapidly reduced by oral folic acid.[33] Doses up to 10 mg have been used,[41] although 5 mg alone[71] or even 1 mg alone[33] or in combination with cyanocobalamin and vitamin B6 may be effective.[35] The lowest effective dose has not yet been established. Such effects may be short-lived if treatment is not continued: in one study homocysteine concentrations returned to baseline levels in 10 weeks following cessation of folic acid therapy.[70]

In theory, vitamin B12, a remethylation cofactor rather than a cosubstrate, might be expected to produce little if any reduction in homocysteine levels unless there is frank cobalamin deficiency. In a limited number of studies, it did not reduce homocysteine levels in normal subjects[71] or in patients with renal disease.[70] Administration of vitamin B6 alone does not reduce fasting homocysteine concentrations in normal subjects[71] or in patients with renal disease.[70]

Folic acid can also reduce plasma homocysteine concentrations in patients with peripheral vascular and cerebrovascular disease.[41] Vitamin B6 may have similar effects but only following methionine loading.[40,41,72]

Recently, various combinations of vitamin B6 and B12 and folic acid have reduced homocysteine concentrations in patients with coronary artery disease.[73,74] A response to vitamin B6 therapy in these patients might be due to the presence of an underlying cystathionine synthase abnormality or to a correction of an underlying pyridoxine deficiency. These effects may have enormous potential therapeutic relevance, and vitamin intervention studies are now being considered in patients with coronary artery disease.

THE FUTURE

Abundant evidence from cross-sectional and clinical case-control studies now suggests that homocysteine is an independent risk factor for vascular disease and is not a proxy for other risk factors. However, many areas still require clarification. Although the relative risk of vascular disease in people with hyperhomocysteinemia appears raised, interrelationships with other risk factors are still unclear and the possibility of synergy has not been excluded.

Although hyperhomocysteinemia may be a risk factor in men, there are limited data on its prevalence and its relationship with other risk factors in women. In one study, women with coronary disease had higher homocysteine levels than did controls.[39] In addition, greater odds ratios for carotid thickening have been seen in women compared with men with increasing quintiles of plasma homocysteine.[75] Also unclear is the role of hyperhomocysteinemia in the elderly and in different ethnic groups, including African-Americans. In one study, no significant elevation in the odds ratios for carotid wall thickening was seen in blacks,[75] although the numbers were small.

The role of homocysteine in the vascular disease of renal failure should be further explored in view of the severe vascular disease and the high homocysteine levels seen in these patients.[76] The association between homocysteine concentrations and the severity of associated vascular diseases also needs further investigation, as some studies[56,59,61,75] have reported that the risk of vascular disease rises with plasma homocysteine concentration.

Recently, increases in plasma homocysteine concentration of almost 70% following cardiac transplantation have been reported.[77] Concentrations of circulating folic acid and vitamin B12 fell significantly in these patients. The underlying mechanism for these marked changes and their possible relationship to the subsequent development of coronary artery disease after transplantation will require additional prospective study. Further investigation of the relationship between homocysteine and thrombotic

tendencies in patients with vascular disease may provide new insights into platelet activation, as homozygous homocystinuria has been associated with increased thromboxane A2 production.[15]

In spite of the observation of high circulating homocysteine concentrations in patients with vascular disease, the underlying mechanisms remain obscure. There is limited evidence that cystathionine synthase deficiency is present in some patients with vascular disease.[40,42,58] The relationship of the activity of cystathionine synthase to the other determinants of transsulfuration and remethylation such as vitamin cofactors is still unclear, and it is uncertain whether the lower vitamin concentrations reported by some authors are secondary to this or have a dietary origin. Other enzyme abnormalities may be important: a thermolabile variant of methylenetetrahydrofolate reductase has recently been reported in patients with coronary artery disease and may be associated with high homocysteine concentrations.[78,79]

Cystathionine synthase deficiency or other hereditary enzyme or cofactor abnormalities could at least partly explain why a positive family history is a risk factor for vascular disease. Genetic mechanisms may be more important in younger patients with coronary artery disease,[40,42,62] while nutritional deficiency may predominate in the elderly.[80] In one study of men with premature coronary artery disease, as many as 14% had familial hyperhomocysteinemia.[81] Genetic or enzymatic screening for such hereditary abnormalities could identify children at increased risk of developing premature vascular disease later in life. The complementary DNA sequence of human cystathionine synthase has been elucidated,[82] and a defect in the gene coding for cystathionine synthase resulting in replacement of glycine 307 by serine in the protein has been reported in patients with homocystinuria.[83] Recently, c-DNA for human methylenetetrahydrofolate reductase has been isolated and two mutations identified in patients with a deficiency of this enzyme.[84] This may be of great importance because of the reports of abnormalities of this enzyme in patients with coronary artery disease.[78,79] The hereditary aspects of homocystinuria have been reviewed elsewhere.[85] Multiple abnormalities of cystathionine synthase may explain the lower enzyme activity in patients with vascular disease, and the abnormalities of methylenetetrahydrofolate reductase require further investigation in these patients.

IMPLICATIONS FOR CLINICIANS

As for the relevance of the homocysteine-vascular disease link to today's practicing clinicians, several key issues need to be resolved. First, is there any merit in measuring homocysteine concentrations in patients with vascular disease? And if so, which test should be used? The current standard is the total fasting plasma homocysteine concentration test, widely used by investigators and certainly less cumbersome than a formal methionine-loading test. However, the fasting test may not confer any advantage over a random sample,[46] although this too requires further study.

Second, the precise plasma homocysteine concentration at which the risk of vascular disease increases remains unclear: there is considerable overlap between values in patients with coronary artery disease and controls. As with hypercholesterolemia, values now considered normal may eventually be considered high.

Third, in spite of the possible effectiveness of pyridoxine in the prevention of thromboembolism in patients with homozygous homocystinuria, there is no controlled evidence that reducing homocysteine concentrations prevents or slows coronary artery disease or influences outcome. Measuring plasma homocysteine concentrations may therefore be somewhat academic until primary and secondary prevention studies have been performed. Such studies will first require the evaluation of the efficacy of different combinations and doses of supplemental vitamin therapies in reducing homocysteine concentrations.

SUMMARY

Homocysteine has adverse effects on endothelium, platelets, and clotting factors. In clinical studies, elevated concentrations have been associated with premature vascular disease and with a greater frequency of acute cardiovascular episodes, including myocardial infarction.[47,56,57] Vitamin therapy, which lowers the plasma homocysteine concentration, is simple, nontoxic, naturally occurring, and has a wide therapeutic index. Together, these observations make a compelling argument for intervention studies in patients with coronary heart disease. Indeed, preliminary investigations are underway in patients with peripheral vascular disease. Such studies in cardiac patients must now focus on progres-

sion and regression of disease and on prognosis. Although such a trial would be expensive and pose a logistic challenge, it would be worthwhile if treatment actually decreases mortality. Many of these exciting areas are currently under investigation at the Cleveland Clinic, with important implications for the fields of vascular medicine, biochemistry, nutrition, epidemiology, and public health.

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