Review Article

Mechanisms of Disease

FRANKLIN H. EPSTEIN, M.D., *Editor*

HOMOCYSTEINE AND ATHEROTHROMBOSIS

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IN 1969, McCully made the clinical observation linking elevated plasma homocyst(e)ine concentrations with vascular disease.[1] He reported autopsy evidence of extensive arterial thrombosis and atherosclerosis in two children with elevated plasma homocyst(e)ine concentrations and homocystinuria. On the basis of this observation, he proposed that elevated plasma homocyst(e)ine (hyperhomocyst(e)inemia) can cause atherosclerotic vascular disease. The term "homocyst(e)ine" is used to define the combined pool of homocysteine, homocystine, mixed disulfides involving homocysteine, and homocysteine thiolactone found in the plasma of patients with hyperhomocyst(e)inemia.

Subsequent investigations have confirmed McCully's hypothesis, and it has recently become clear that hyperhomocyst(e)inemia is an independent risk factor for atherosclerosis and atherothrombosis. Although severe hyperhomocyst(e)inemia is rare, mild hyperhomocyst(e)inemia occurs in approximately 5 to 7 percent of the general population.[2,3] Patients with mild hyperhomocyst(e)inemia have none of the clinical signs of severe hyperhomocyst(e)inemia or homocystinuria and are typically asymptomatic until the third or fourth decade of life when premature coronary artery disease develops, as well as recurrent arterial and venous thrombosis. Abundant epidemiologic evidence has demonstrated that the presence of mild hyperhomocyst(e)inemia is an independent risk factor for atherosclerosis in the coronary, cerebral, and peripheral vasculature (see below).[4,5] Although the molecular mechanism by which homocyst(e)ine or a related metabolite promotes atherothrombosis is unknown, the epidemiologic evidence of the association of hyperhomocyst(e)inemia with atherothrombotic vascular disease is convincing. In this review, we will evaluate the evidence of a relation between elevated plasma homocyst(e)ine concentrations and vascular disease. Potential mechanisms for this effect are also discussed.

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HOMOCYST(E)INE METABOLISM

Homocyst(e)ine is a sulfur-containing amino acid formed during the metabolism of methionine. Homocyst(e)ine is metabolized by one of two pathways: remethylation and transsulfuration (Fig. 1). In the remethylation cycle, homocyst(e)ine is salvaged by the acquisition of a methyl group in a reaction catalyzed by methionine synthase.[6] Vitamin B_{12} (cobalamin) is an essential cofactor for methionine synthase, N^5-methyl-tetrahydrofolate is the methyl donor in this reaction, and N^5,N^{10}-methylenetetrahydrofolate reductase functions as a catalyst in the remethylation process.

Under conditions in which an excess of methionine is present or cysteine synthesis is required, homocyst(e)ine enters the transsulfuration pathway. In this pathway, homocyst(e)ine condenses with serine to form cystathionine in a reaction catalyzed by the vitamin B_6–dependent enzyme cystathionine β-synthase.[6] Cystathionine is subsequently hydrolyzed to form cysteine, which may in turn be incorporated into glutathione or further metabolized to sulfate and excreted in the urine.[7]

MEASUREMENT OF PLASMA HOMOCYST(E)INE

The majority of the clinical studies involving homocyst(e)ine have relied on the measurement of total plasma homocyst(e)ine, which includes homocysteine, mixed disulfides involving homocysteine, homocysteine thiolactone, free homocysteine, and protein-bound homocysteine. Protein-bound (i.e., disulfide-linked) homocysteine accounts for 70 to 80 percent of the total pool.[8] Normal total plasma homocyst(e)ine concentrations range from 5 to 15 μmol per liter in the fasting state.[6,9] Kang and coworkers have classified hyperhomocyst(e)inemia as moderate (homocyst(e)ine concentration, 15 to 30 μmol per liter), intermediate (>30 to 100 μmol per liter), and severe (>100 μmol per liter)[4] on the basis of concentrations measured during fasting.

An oral dose of methionine (100 mg per kilogram of body weight) can be given to persons with sus-

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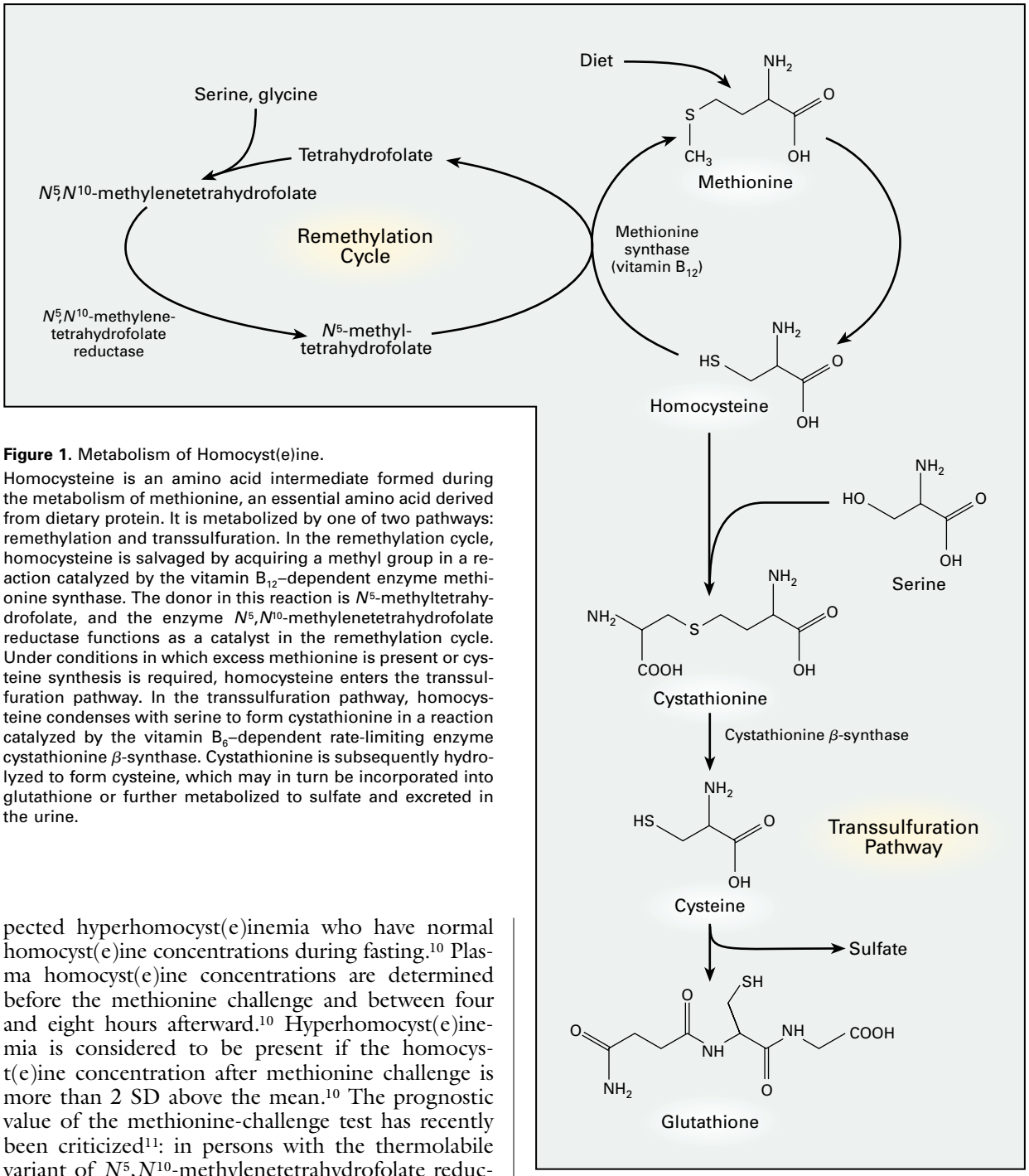

Figure 1. Metabolism of Homocyst(e)ine.

Homocysteine is an amino acid intermediate formed during the metabolism of methionine, an essential amino acid derived from dietary protein. It is metabolized by one of two pathways: remethylation and transsulfuration. In the remethylation cycle, homocysteine is salvaged by acquiring a methyl group in a reaction catalyzed by the vitamin B_{12}–dependent enzyme methionine synthase. The donor in this reaction is N^5-methyltetrahydrofolate, and the enzyme N^5,N^{10}-methylenetetrahydrofolate reductase functions as a catalyst in the remethylation cycle. Under conditions in which excess methionine is present or cysteine synthesis is required, homocysteine enters the transsulfuration pathway. In the transsulfuration pathway, homocysteine condenses with serine to form cystathionine in a reaction catalyzed by the vitamin B_6–dependent rate-limiting enzyme cystathionine β-synthase. Cystathionine is subsequently hydrolyzed to form cysteine, which may in turn be incorporated into glutathione or further metabolized to sulfate and excreted in the urine.

pected hyperhomocyst(e)inemia who have normal homocyst(e)ine concentrations during fasting.[10] Plasma homocyst(e)ine concentrations are determined before the methionine challenge and between four and eight hours afterward.[10] Hyperhomocyst(e)inemia is considered to be present if the homocyst(e)ine concentration after methionine challenge is more than 2 SD above the mean.[10] The prognostic value of the methionine-challenge test has recently been criticized[11]: in persons with the thermolabile variant of N^5,N^{10}-methylenetetrahydrofolate reductase, there was only a weak association between plasma homocyst(e)ine concentrations after methionine challenge and premature coronary heart disease (odds ratio, 1.7; $P=0.12$), whereas there was a significant association between plasma homocyst(e)ine concentrations during fasting and premature coronary heart disease (odds ratio, 2.0; $P=0.04$). These authors concluded that this enzyme regulates basal homocyst(e)ine concentrations, and thus, its activity cannot be adequately assessed by a methionine-challenge test. By contrast, enzymes in the transsulfuration pathway are responsible for reversing transient, postprandial increases in the homocyst(e)ine concentration, and their activities can be evaluated by a methionine-challenge test.

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GENETIC DEFECTS IN HOMOCYSTEINE METABOLISM

Elevations in plasma homocyst(e)ine are typically caused either by genetic defects in the enzymes involved in homocysteine metabolism or by nutritional deficiencies in vitamin cofactors. Homocystinuria and severe hyperhomocyst(e)inemia are caused by rare inborn errors of metabolism resulting in marked elevations of plasma and urine homocyst(e)ine concentrations. Cystathionine β-synthase deficiency is the most common genetic cause of severe hyperhomocyst(e)inemia. The homozygous form of this disease — congenital homocystinuria — can be associated with plasma homocyst(e)ine concentrations of up to 400 μmol per liter during fasting.[12] The homozygous trait is rare (occurring in 1 in 200,000 births), and clinical manifestations include ectopia lentis, skeletal deformities, mental retardation, thromboembolism, and severe, premature atherosclerosis.[12] Atherothrombotic complications frequently develop in young adulthood in homozygotes and are often fatal, as first shown in a study by Carey and colleagues as early as 1968.[13] Mudd and colleagues[14] have estimated that approximately 50 percent of untreated patients with homocystinuria will have a thromboembolic event before the age of 30 and that overall, the disease-related mortality is approximately 20 percent. Heterozygotes typically have much less marked hyperhomocyst(e)inemia, with plasma homocyst(e)ine concentrations in the range of 20 to 40 μmol per liter, approximately two to four times greater than the normal concentration of homocyst(e)ine in plasma.[13,15-17]

A homozygous deficiency of N^5,N^{10}-methylenetetrahydrofolate reductase, the enzyme involved in the vitamin B_{12}–dependent remethylation of homocysteine to methionine, may also lead to severe hyperhomocyst(e)inemia.[18] Patients with this type of deficiency tend to have a worse prognosis than those with cystathionine β-synthase deficiency, in part because of the complete lack of effective therapy.[19,20] In addition, Kang and colleagues[21] have reported a thermolabile variant of N^5,N^{10}-methylenetetrahydrofolate reductase that is caused by a point mutation (C677T) in the coding region for the N^5,N^{10}-methylenetetrahydrofolate binding site, leading to the substitution of valine for alanine.[22] This mutation was found in 38 percent of French Canadians and 5 to 15 percent of the general population in Canada and correlated with elevated plasma homocyst(e)ine concentrations.[22,23] Although this variant of the N^5,N^{10}-methylenetetrahydrofolate reductase gene is quite common, it does not appear to be a significant, independent risk factor for atherothrombotic vascular disease.[24-26] Persons who are homozygous for this mutation appear to have an exaggerated hyperhomocyst(e)inemic response to the depletion of folic acid and with folic acid depletion may be at increased risk for vascular disease.[26] Other abnormalities of the remethylation cycle that are associated with hyperhomocyst(e)inemia include methionine synthase deficiency and disorders of vitamin B_{12} metabolism that impair methionine synthase activity.

NUTRITIONAL DEFICIENCIES CAUSING HYPERHOMOCYST(E)INEMIA

Nutritional deficiencies in the vitamin cofactors (folate, vitamin B_{12}, and vitamin B_6) required for homocysteine metabolism may promote hyperhomocyst(e)inemia. Markedly elevated homocyst(e)ine concentrations have been observed in patients with nutritional deficiencies of the essential cofactor vitamin B_{12}[27] and the cosubstrate folate.[28,29] Negative correlations between serum vitamin B_{12}, folate, and vitamin B_6 concentrations and plasma homocyst(e)ine concentrations have been observed in normal subjects.[30] Selhub and colleagues[30] have suggested that inadequate plasma concentrations of one or more B vitamins are contributing factors in approximately two thirds of all cases of hyperhomocyst(e)inemia. Vitamin supplementation can normalize high homocyst(e)ine concentrations (see below); however, it remains to be determined whether normalizing homocyst(e)ine concentrations will improve cardiovascular morbidity and mortality.

OTHER CAUSES OF HYPERHOMOCYST(E)INEMIA

A number of other factors influence homocyst(e)ine metabolism, including several disease states and medications. Plasma homocyst(e)ine concentrations increase with elevations in creatinine and are typically elevated in chronic renal failure, often approaching concentrations that are up to four times the normal value.[31,32] Although plasma homocyst(e)ine concentrations often decrease after dialysis,[32] it is unclear whether the elevation in homocyst(e)ine observed in end-stage renal disease is due to impaired metabolism or to reduced excretion. The presence of elevated plasma homocyst(e)ine concentrations may partially explain the observed acceleration of atherosclerosis in end-stage renal disease.

A number of reports have linked hyperhomocyst(e)inemia to hypothyroidism,[3] suggesting a potential mechanism for the higher incidence of vascular disease observed in patients with hypothyroidism. Hyperhomocyst(e)inemia has also been reported in patients with pernicious anemia, and elevated plasma homocyst(e)ine concentrations are helpful in diagnosing this disorder. In one study of 434 patients with cobalamin deficiency, approximately 96 percent had serum homocyst(e)ine concentrations that were more than 3 SD above the

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mean.[33] It is unclear whether these patients are at increased risk for vascular events.

Elevated homocyst(e)ine concentrations have been reported in association with several types of carcinoma, including breast, ovarian, and pancreatic cancer.[34] Transformed cells in culture are unable to use homocyst(e)ine, and it has been suggested that proliferating tumor cells may also be incapable of metabolizing endogenous homocysteine.[2,34] In addition, acute lymphoblastic leukemia is associated with marked elevations in plasma homocyst(e)ine; after chemotherapy for this disorder, homocyst(e)ine concentrations decrease dramatically.[34,35]

Several drugs and toxins increase plasma homocyst(e)ine concentrations. Methotrexate depletes folate, the cosubstrate for methionine synthase, and causes a transient increase in plasma homocyst(e)ine concentrations.[2,36] Phenytoin also interferes with folate metabolism and may cause mild hyperhomocyst(e)inemia.[2,36] Theophylline, a phosphodiesterase inhibitor, may cause hyperhomocyst(e)inemia by antagonizing the synthesis of pyridoxal phosphate (vitamin B_6).[37] Cigarette smoking also interferes with the synthesis of pyridoxal phosphate,[38] and it has recently been reported that smokers have significantly lower pyridoxal phosphate concentrations than nonsmokers.[39] These results suggest another important mechanism whereby smoking may promote atherogenesis.

ASSOCIATION BETWEEN HYPERHOMOCYST(E)INEMIA AND ATHEROSCLEROSIS

Since McCully hypothesized that elevated plasma homocyst(e)ine concentrations could cause atherosclerosis, abundant epidemiologic evidence from more than 20 case–control and cross-sectional studies involving over 2000 patients[36,40] has validated this relation. Boers and colleagues[41] screened 75 patients with premature atherosclerotic vascular disease for hyperhomocyst(e)inemia using methionine challenge and found that nearly one third of all patients with cerebrovascular disease and peripheral vascular disease had hyperhomocyst(e)inemia. Clarke and colleagues[42] subsequently measured homocyst(e)ine concentrations after methionine loading in a cohort of men with premature vascular disease and normal controls and demonstrated that 42 percent of patients with cerebrovascular disease, 28 percent of patients with peripheral vascular disease, and 30 percent of patients with coronary artery disease had hyperhomocyst(e)inemia. Clarke et al. also found that the relative risk of coronary artery disease in patients with hyperhomocyst(e)inemia was approximately 24 times that in controls.

Two large, prospective studies have assessed the risk of coronary artery disease in patients with hyperhomocyst(e)inemia. In the Physicians' Health Study, 14,916 male physicians without known atherosclerosis had an initial homocyst(e)ine measurement and were prospectively followed for an average of five years.[5] Men with plasma homocyst(e)ine concentrations that were 12 percent above the upper limit of normal had approximately a threefold increase in the risk of myocardial infarction, as compared with those with lower levels, even after correction for other risk factors. The authors estimated that 7 percent of the 271 observed myocardial infarctions could be attributed to hyperhomocyst(e)inemia. The prospective design of the study eliminated the possibility that atherosclerosis itself may have altered homocyst(e)ine concentrations. The prospective Trømso study[43] reported similar results, and several other prospective studies have consistently indicated that hyperhomocyst(e)inemia is an independent risk factor for vascular disease.[44-46]

Selhub and colleagues[47] have recently demonstrated that the prevalence of carotid-artery stenosis increases with increasing plasma concentrations of homocyst(e)ine. In a cross-sectional study of 1041 elderly subjects in the Framingham Heart Study, they found a strong association between elevated homocyst(e)ine concentrations and occlusive vascular disease that remained even after adjustment for other conventional coronary risk factors. There was a graded, rather than a threshold, relation between plasma homocyst(e)ine and the risk of carotid stenosis. The risk of carotid stenosis was increased even at lower plasma concentrations of homocyst(e)ine (between 11.4 and 14.3 μmol per liter) that had previously been considered to be normal. Malinow and colleagues[48] reported similar results in an earlier study. A graded response has also been demonstrated between homocyst(e)ine concentrations and the risk of coronary artery disease or cerebrovascular accident.[5,49-51]

Graham and coworkers[52] recently measured plasma homocyst(e)ine concentrations in 750 patients with atherosclerosis and 800 normal subjects. There was a statistically significant difference in plasma homocyst(e)ine concentrations during fasting between patients and controls (11.25 μmol per liter vs. 9.73 μmol per liter, $P<0.001$), and a methionine challenge revealed that an additional 27 percent of patients had hyperhomocyst(e)inemia. Interestingly, an elevated plasma homocyst(e)ine concentration conferred an independent risk of vascular disease similar to that of smoking or hypercholesterolemia and also had a multiplicative effect on risk among cigarette smokers and patients with hypertension.[52] The authors therefore suggested that controlling hypertension and smoking may be particularly important in patients with hyperhomocyst(e)inemia.

Nygård and colleagues[53] recently reported a prospective study involving 587 patients with angiographically documented coronary artery disease. Baseline homocyst(e)ine measurements were obtained,

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and patients were followed for a median of 4.6 years, during which time 10.9 percent of them died. These investigators found a strong, graded association between plasma homocyst(e)ine concentrations and overall mortality. The relation between homocyst(e)ine and mortality was strongest for total homocyst(e)ine concentrations above 15 μmol per liter. The adjusted mortality ratio was 1.6 for patients with homocyst(e)ine concentrations of 15 μmol per liter, as compared with those with values of 10 μmol per liter. However, total homocyst(e)ine concentrations were only weakly associated with the extent of coronary artery disease in this study. This study and other key studies designed to evaluate the relation between plasma homocyst(e)ine concentrations and atherothrombotic risk are summarized in Table 1.

In a recent meta-analysis, Boushey and colleagues[54] estimated that 10 percent of the risk of coronary artery disease in the general population is attributable to homocyst(e)ine. They reported that an increase of 5 μmol per liter in the plasma homocyst(e)ine concentration raises the risk of coronary artery disease by as much as an increase of 20 mg per deciliter (0.52 mmol per liter) in the cholesterol concentration. They suggested that increasing folate consumption by approximately 200 μg per day would reduce total homocyst(e)ine concentrations by approximately 4 μmol per liter, a reduction that could potentially have a major effect on cardiovascular mortality. Randomized clinical trials will be necessary to demonstrate the clinical utility of this strategy. Importantly, the current fortification program will increase folate consumption by approximately half this amount, and it remains to be seen whether this dietary supplementation of folate will affect the prevalence of coronary heart disease in the general population.

Den Heijer and colleagues[55] have demonstrated that mild hyperhomocyst(e)inemia is also an independent risk factor for venous thromboembolism. They found a marked increase in the risk of venous thrombosis at the highest plasma homocyst(e)ine concentrations. A plasma homocyst(e)ine concentration of more than 22 μmol per liter increased the matched odds ratio for deep venous thrombosis to 4.0. It is unknown whether homocyst(e)ine-lowering therapy reduces the risk of venous thrombosis in patients with such high concentrations. Recently, Ridker and associates[56] showed that the combination of hyperhomocyst(e)inemia and factor V Leiden further increases the relative risk of venous thromboembolism up to 3.6-fold.

TREATMENT OF HYPERHOMOCYST(E)INEMIA

The treatment of hyperhomocyst(e)inemia varies with the underlying cause; however, vitamin supplementation (with folic acid, pyridoxine, and vitamin B_{12}) is generally effective in reducing homocyst(e)ine concentrations. The minimal effective doses of folic acid and pyridoxine have not yet been determined. In most patients, small doses of folate (1 to 5 mg per day) rapidly decrease homocyst(e)ine concentrations.[57] Folic acid alone, folic acid combined with vitamins B_{12} and B_6, and vitamins B_6 and B_{12} have all been shown to reduce homocyst(e)ine con-

TABLE 1. MAJOR OBSERVATIONAL STUDIES OF HOMOCYST(E)INE AS A RISK FACTOR FOR ATHEROTHROMBOTIC DISEASE.*

REFERENCE	STUDY DESIGN	MAIN END POINT	MEAN HOMOCYST(E)INE CONCENTRATION		RELATIVE RISK (95% CI)	P VALUE
			CASE PATIENTS	CONTROLS		
			μmol/liter			
Taylor et al.[46]	Case–control	Progression of CAD, CVD, or PVD	14.37±6.89	10.10±2.16	NA	<0.05
Graham et al.[52]	Case–control	CAD, CVD, PVD	11.25	9.73	1.9 (1.4–2.4)	<0.001
Stampfer et al.[5]	Nested case–control	Acute MI or death from cardiovascular causes	11.1±4.0	10.5±2.8	3.4 (1.3–8.8)	0.026
Verhoef et al.[45]	Nested case–control	Ischemic CVA	11.1±4.0	10.6±3.4	1.2 (0.7–2.0)	0.12
Arnesen et al.[43]	Nested case–control	Development of CAD	12.7±4.7	11.3±3.7	1.32 (1.05–1.65)	0.002
Perry et al.[51]	Nested case–control	CVA	13.7	11.9	NA	0.004
Nygård et al.[53]	Prospective, observational	Overall mortality, extent of CAD	Males, 11.4 Females, 10.5	NA	NA	0.005† 0.02‡

*Plus–minus values are means ±SD. CI denotes confidence interval, CAD coronary artery disease, CVD cerebrovascular disease, PVD peripheral vascular disease, NA not available, MI myocardial infarction, and CVA cerebrovascular accident.

†The P value is for the trend in the relative risk of stroke as a function of quartiles of plasma homocyst(e)ine concentration.

‡The P value is for the trend in the relative risk of death as a function of plasma homocyst(e)ine concentration.

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centrations.[58] Normalization of the plasma homocyst(e)ine concentration usually occurs within four to six weeks after the initiation of therapy, but may occur in as little as two weeks. Interestingly, the reduction in mortality from cardiovascular causes since 1960 has been correlated with the increase in vitamin B_6 supplementation in the food supply.[3]

PATHOPHYSIOLOGIC MECHANISMS OF HYPERHOMOCYST(E)INEMIA

Experimental evidence suggests that the atherogenic propensity associated with hyperhomocyst(e)inemia results from endothelial dysfunction and injury followed by platelet activation and thrombus formation. Studies in humans and animals demonstrate that homocyst(e)ine-induced atherosclerosis is characterized by substantial platelet accumulation and platelet-rich thrombus formation in areas of endothelial injury.[59-61] Harker and colleagues have proposed that homocyst(e)ine-induced endothelial injury exposes the subendothelial matrix, which in turn leads to platelet activation.[59,60] Lentz and colleagues[62] have demonstrated that diet-induced hyperhomocyst(e)inemia in primates leads to impaired vasomotor regulation in vivo and endothelial antithrombotic function ex vivo. These findings are supported by the work of Celermajer and colleagues,[63] who demonstrated impaired endothelium-dependent vasodilation, and also by van den Berg and colleagues,[64] who demonstrated impaired endothelial anticoagulant function in young patients with hyperhomocyst(e)inemia and peripheral vascular disease. Although the exact mechanism of endothelial dysfunction is unknown, there is growing evidence that homocyst(e)ine exerts its effects by promoting oxidative damage.

Homocyst(e)ine is rapidly auto-oxidized when added to plasma, forming homocystine, mixed disulfides, and homocysteine thiolactone (Fig. 2).[65-67] Potent reactive oxygen species, including superoxide and hydrogen peroxide, are produced during the auto-oxidation of homocyst(e)ine, and hydrogen peroxide (along with the hydroxyl radical), in particular, has been implicated in the vascular toxicity of hyperhomocyst(e)inemia.[68] There is extensive evidence that homocyst(e)ine-induced endothelial-cell injury in vitro is largely due to the generation of hydrogen peroxide.[69-71] Harker and colleagues[59] have proposed that homocyst(e)ine-induced endothelial-cell injury mediated by hydrogen peroxide exposes the underlying matrix and smooth-muscle cells, which in turn proliferate and promote the activation of platelets and leukocytes.

Auto-oxidation of homocyst(e)ine produces other cytotoxic reactive oxygen species, including the superoxide anion radical and hydroxyl radical.[72,73] Superoxide-dependent formation of the hydroxyl radical has been shown to initiate lipid peroxidation,[73]

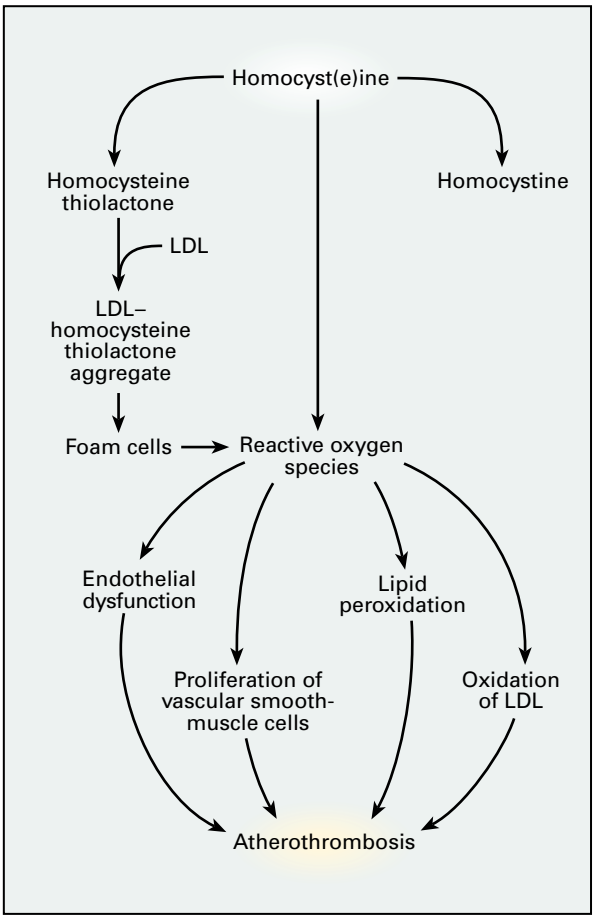

Figure 2. Postulated Adverse Vascular Effects of Homocyst(e)ine.

The postulated effects involve oxidative damage to vascular endothelial cells and increased proliferation of vascular smooth-muscle cells after oxidative metabolism of homocysteine to homocystine and homocysteine thiolactone. Oxidative modification of low-density lipoprotein (LDL) promotes the formation of foam cells, which in turn yields another source of reactive oxygen species.

an effect that occurs at the level of the endothelial plasma membrane and within lipoprotein particles.[74,75] Homocyst(e)ine auto-oxidation has been shown to support the oxidation of low-density lipoprotein through the generation of the superoxide anion radical.[74,76]

Although the precise molecular mechanism is unknown, homocyst(e)ine causes endothelial dysfunction at several levels. Homocyst(e)ine alters the normal antithrombotic phenotype of the endothelium by enhancing the activities of factor XII[77] and factor V[78] and depressing the activation of protein C.[79] Homocyst(e)ine also inhibits the expression of thrombomodulin,[80] induces the expression of tissue factor,[81] and suppresses the expression of heparan sulfate by the endothelium.[82] All of these effects ultimately

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facilitate the formation of thrombin and create a prothrombotic environment.

The production of endothelial-derived nitric oxide is also adversely affected by homocyst(e)ine. Our group[66] has previously shown that normal endothelial cells detoxify homocyst(e)ine by releasing nitric oxide, which combines with homocysteine in the presence of oxygen to form *S*-nitroso-homocysteine. Nitrosation of the sulfhydryl group of homocysteine inhibits sulfhydryl-dependent generation of hydrogen peroxide.[66] *S*-nitroso-homocysteine is also a potent platelet inhibitor and vasodilator.[83] This protective effect of nitric oxide is eventually compromised as long-term exposure to hyperhomocyst(e)inemia damages the endothelium sufficiently to limit nitric oxide production. Impaired endothelial production of nitric oxide leaves the endothelium vulnerable to unopposed homocyst(e)ine-mediated oxidative injury.[66] Homocyst(e)ine may also decrease the bioavailability of nitric oxide by impairing its synthesis.[68,75] Homocyst(e)ine promotes lipid peroxidation, which may subsequently decrease the expression of endothelial nitric oxide synthase and directly degrade nitric oxide.[84-86] Our group has recently shown that homocysteine (but not cysteine) suppresses the expression of cellular glutathione peroxidase by endothelial cells, and this effect promotes lipid peroxidation by the reactive oxygen species elaborated during the oxidation of homocysteine.[87]

In addition to promoting atherosclerosis through endothelial injury or dysfunction, homocyst(e)ine is also a potent mitogen for vascular smooth-muscle cells. Harker and colleagues[88] demonstrated that infusion of homocyst(e)ine into baboons results in the formation of atheromata. Exposure to homocyst(e)ine leads to a marked increase in vascular smooth-muscle proliferation in vitro, an effect that is due in part to an increase in the expression of messenger RNA of cyclin D1 and cyclin A.[89] Tsai and coworkers[90] have proposed that homocyst(e)ine promotes atherogenesis specifically by inducing the proliferation of vascular smooth-muscle cells.

We have recently demonstrated that homocyst(e)ine increases nitric oxide production in vascular smooth-muscle cells by activating the transcription factor NF-κB.[91] It appears that NF-κB is activated by a homocyst(e)ine-generated reactive oxygen species.[91] Since NF-κB/rel activity is essential for the proliferation of vascular smooth-muscle cells,[92] these data suggest that homocyst(e)ine-mediated activation of NF-κB contributes to the mitogenic effect of homocyst(e)ine.

Homocyst(e)ine also directly damages the vascular matrix by affecting the biochemical and biosynthetic functions of vascular cells. Homocysteine thiolactone, a highly reactive anhydrous byproduct of homocysteine oxidation, combines with low-density lipoprotein to form aggregates that are taken up by intimal macrophages and incorporated into foam cells within nascent atheromatous plaques.[93] There is, however, some doubt that the thiolactone can form in sufficient concentrations in vivo to evoke these effects. Recently, Jakubowski showed that cells deficient in cystathionine β-synthase produce more homocysteine thiolactone in culture than normal cells and that the thiolactone is incorporated into cellular and secreted proteins through lysine acylation by the activated carboxyl group of the thiolactone.[94] McCully[3] has suggested that in this microenvironment homocysteine thiolactone facilitates the conversion of mitochondrial thioretinaco ozonide to thioco, thereby impairing oxidative phosphorylation and promoting the proliferation and fibrosis of smooth muscles.[95-97] This homocyst(e)ine-induced disturbance in oxidative metabolism also leads to overproduction of oxidative radicals that subsequently induce intimal injury, activate elastase, and increase calcium deposition.[96,98] Homocyst(e)ine may also contribute to the deposition of sulfated glycosaminoglycan in the matrix; it appears that the sulfur group of homocysteine thiolactone is incorporated into phosphoadenosine phosphosulfate, which ultimately leads to the formation of sulfated glycosaminoglycans.[98]

The recently observed multiplicative increase in the risk of vascular disease in the presence of traditional risk factors and hyperhomocyst(e)inemia[52] may in part be related to the effect of homocyst(e)ine on lipid peroxidation. The vascular cytotoxicity of oxidized low-density lipoprotein has been linked to its content of lipid peroxidation products.[99,100] Homocyst(e)ine increases the formation of highly atherogenic oxycholesterols, increases lipid peroxidation, and increases the oxidation of low-density lipoprotein in vitro.[101,102] These observations suggest a potential role for antioxidant therapy in ameliorating homocyst(e)ine-dependent oxidative vascular injury; however, this therapeutic approach has not yet been tested in prospective clinical trials.

CONCLUSIONS

Multiple prospective and case–control studies have shown that a moderately elevated plasma homocysteine concentration is an independent risk factor for atherothrombotic vascular disease. Homocysteine concentrations are consistently higher in patients with peripheral, cerebrovascular, and coronary artery disease than in those without such diseases. Homocyst(e)ine promotes atherothrombogenesis by a variety of mechanisms; however, it is not yet clear whether homocysteine itself or a related metabolite or cofactor is primarily responsible for the atherothrombogenic effects of hyperhomocyst(e)inemia in vivo. Before advocating widespread screening of patients with atherosclerotic vascular disease, we must have a clearer understanding of the

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clinical efficacy of potential therapeutic interventions. Vitamin supplementation decreases or even normalizes plasma homocyst(e)ine concentrations in most cases. Prospective, randomized clinical trials, however, will be necessary to determine the effect of vitamin supplementation on cardiovascular morbidity and mortality.

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