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Homocysteine, a Risk Factor for Cardiovascular Disease

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Abstract. Fasting hyperhomocysteinemia is an independent risk factor for coronary artery disease, stroke, peripheral vascular atherosclerosis, and for arterial and venous thromboembolism. The risk for cardiovascular disease with homocysteine is similar to conventional risk factors. The interaction of hyperhomocysteinemia with hypertension and smoking is strong and the combined effect is more than multiplicative. The combined effect of homocysteine and cholesterol is additive. Homocysteine produces atherosclerosis, thromboembolism, and vascular endothelial cell injury. Vascular dysfunction produced by homocysteine may be due to endothelial cell damage. Homocysteinemia-induced atherosclerosis is probably due to various factors including endothelial cell injury, inability to sustain S-nitroso-homocysteine formation because of imbalance between production of nitric oxide by dysfunctional endothelium and homocysteine, smooth muscle cell proliferation, and thromboembolism. There is strong evidence that endothelial cell injury is associated with oxidative stress produced by homocysteine. Hyperhomocysteinemia is associated with numerous conditions, including coronary disease, stroke, peripheral vascular disease (carotid artery and cerebrovascular atherosclerosis), venous thrombosis, renal disease, diabetes mellitus, and organ transplant. Folic acid, vitamin B_{12} and B_6 have been shown to be beneficial in reducing plasma homocysteine levels. Folic acid is specifically very effective, safe and inexpensive.

Introduction

Atherosclerosis is a slowly progressive disease which begins in childhood and does not become manifest until middle-age or later. It can progressively or abruptly interfere with blood flow, particularly through heart and brain, and often causes serious clinical consequences such as heart attack and stroke. Atherosclerosis and its complications, such as myocardial infarction, stroke, and peripheral vascular disease, remain major causes of morbidity and mortality in the Western World. Risk factors for atherosclerosis include: high serum total cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides and lipoprotein, (a) [$LP_{(a)}$], low high-density lipoprotein cholesterol (HDL-C), glucose intolerance, android obesity, cigarette smoking, and hypertension. Infection [1,2] and homocysteinemia [3,4] have emerged as risk factors for atherosclerosis in recent years. There has been an explosion of information on homocysteine as a risk factor for the development of atherosclerosis in the coronary, peripheral and cerebrovascular systems. The prevalence of homocysteinuria varies throughout the world but ranges from 1 in 50,000 to 1 in 100,000 newborns. Patients with homocysteinuria frequently have dislocation of optic lenses resulting in acute glaucoma and diminished visual acuity, mental retardation [5], severe atherosclerosis [3], thromboembolic venous disease [6], pregnancy complications [7], neural tube defects [8] and cognitive impairment in the elderly [9]. There is increasing evidence that hyperhomocysteinemia is a risk factor for cardiovascular disease [10–12]. This review article describes the synthesis and metabolism of homocysteine, causes of hyperhomocysteinemia, mechanism of homocysteinemia-induced atherosclerosis, hyperhomocysteinemia and various cardiovascular diseases, and measures to reduce homocysteine concentrations.

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Synthesis and Metabolism

Homocysteine is a sulfur-containing amino acid derived from methionine and is essential for a number of biochemical processes including metabolism of nucleic acids, fats and high energy bonds. Synthesis and metabolism of homocysteine involves three processes: demethylation, transmethylation, and transsulfuration (Fig. 1).

Demethylation

This process converts methionine to homocysteine through intermediate metabolites, S-adenosylmethionine, and S-adenosylhomocysteine.

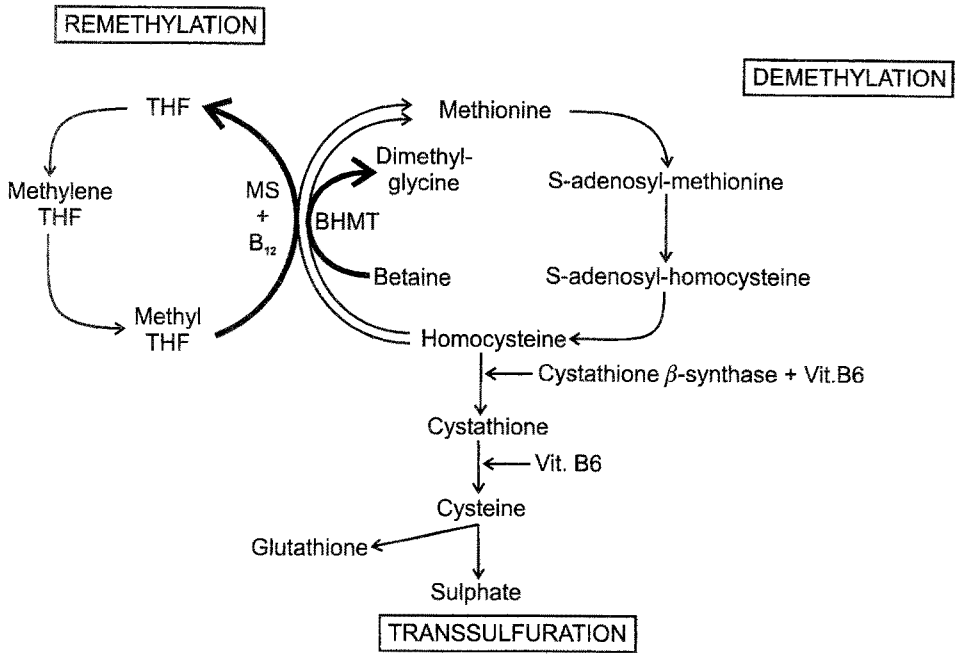

Fig. 1. Metabolism of homocysteine. BHMT, betaine-homocysteine methyltransferase; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; THF, tetrahydrofolate.

Transmethylation

In the transmethylation pathway homocysteine is remethylated to methionine. In the liver, homocysteine is remethylated by betaine-homocysteine methyltransferase which uses betaine as methyl donor [13]. Homocysteine is catalyzed by the methionine synthase which uses vitamin B_{12} as a cofactor and methyltetrahydrofolate as a substrate [14]. Methylenetetrahydrofolate reductase catalyzes the formation of methyltetrahydrofolate [15,16]. In normal metabolism the majority (>50%) of homocysteine is remethylated to methionine requiring folate and vitamin B_{12} [17].

Transsulfuration

In this process homocysteine is irreversibly converted to cysteine. The first reaction in this pathway is catalyzed by vitamin B6-dependent cystathione β-synthase to form cystathionine [14,17]. Cystathionine is hydrolyzed to form cysteine, which in turn is incorporated into glutathione or further metabolized to sulfate and excreted in the urine [18]. This process occurs when excess of methionine is present or cysteine synthesis is required.

Normal Values

The normal fasting range of plasma homocysteine is 5–15 μmoles/L [14]. About 70% of plasma homocysteine is bound to serum protein [19] and is in equilibrium with free homocysteine. Greater than 15 μmoles/L of plasma homocysteine is considered hyperhomocysteinemia [20]. Hyperhomocysteinemia is moderate (15–30 μmoles/L), intermediate (30–100 μmoles/L), and severe (>100 μmoles/L) [20]. Individuals with adequate vitamin status have lower levels of homocysteine. The values for nonsmokers with high folate intake and low coffee consumption are 4.7 to 11.4 μmoles/L and 6.3–13.1 μmoles/L respectively [21]. In children aged 8–12 years the homocysteine levels are about half that of adults. The homocysteine level increases at puberty and the distribution becomes skewed in adults.

Causes of Hyperhomocysteinemia

Causes of homocysteinemia are shown in Table I. They can be due to deficiency in enzymes, or in vitamins, disease states, and drugs. Hyperhomocysteinemia is caused by defect in transsulfuration of homocysteine to cysteine because of a defect in the gene for cystathione or in enzymes (methionine synthase, 5, 10-methylenetetrahydrofolate reductase) for methylation of homocysteine to methionine [36]. Deficiency in vitamin B_6, vitamin B_{12}, and folic acid interferes with transsulfuration and remethylation process and hence there is an increase in the plasma concentration of homocysteine [12,24,25,33].

Females have lower levels of plasma homocysteine than males and it increases with age [37]. Plasma homocysteine levels increase after menopause [38]. Dietary intake of vitamin B_6, vitamin B_{12}, and folic acid is inversely correlated to plasma homocysteine [39]. Smoking and caffeinated coffee consumption increase homocysteine levels but exercise decreases it [40,41]. Chronic high ethanol consumption is associated with an increase in homocysteine levels [42] while moderate consumption is associated with lower levels of homocysteine.

Hyperhomocysteinemia has also been reported in patients with pernicious anemia; and elevated plasma homocysteine concentrations are helpful in the diagnosis of this disorder [43]. Hyperhomocysteinemia is associated with several types of carcinoma including breast, ovary, and pancreas [33]. Theophylline-induced hyperhomocysteinemia may be due to antagonization of synthesis of pyridoxal phosphate (vitamin B_6) [44]. Cigarette smoking-induced hyperhomo-

Table 1. Causes of hyperhomocysteinemia

	References
A. Enzyme deficiency	
Cystathione β-Synthase	22
Methionine Synthase	23
5, 10-methylenetetrahydrofolate reductase	15, 24
B. Vitamin deficiency	
Folic acid	12, 17
Vitamin B_6	24
Vitamin B_{12}	25
C. Drugs	
Inhibitor of dihydrofolate reductase (methotrexate)	26
Folic acid antagonist (phenytoin, carbamazepine)	23, 26
Methionine synthase inhibitor (nitrous oxide)	23 (quoted)
Vitamin B_{12} antagonist (nitrous oxide)	27
Vitamin B_6 antagonist (theophylline, 6-aza-uridine triacetate)	23, 28, 29
L-dopa	30
Cholesterol lowering drugs (cholestyramine, niacin)	31
D. Diseases	
Chronic renal failure	32
Stroke	12
Coronary artery disease	33
Deep venous thrombosis	34
Hypertension, psoriasis and acute lymphoblastic leukemia	23 (quoted)
Hypothyroidism	35

cysteinemia could be due to interference with the synthesis of pyridoxal phosphate [41].

Effect of Homocysteine

Homocysteine produces thromboembolism, atherosclerosis and vascular endothelial cell damage. Homocysteine affects connective tissue, smooth muscle cells, platelets, endothelial cells, the vessel wall, blood lipids, coagulation factors, and nitric oxide. Homocysteine stimulates proliferation of vascular smooth muscle cells in culture [45]. It increases DNA synthesis, growth, and cyclin A gene expression [46] in cultured vascular smooth muscle cells, and cyclin-dependent kinase expression in the aorta of rats [47]. It promotes platelet aggregation [48]. Homocysteine may enhance binding of lipoprotein (a) to fibrin [49]. In high concentrations it activates factor V [50], reduces protein C activation [51], inactivates the cofactor activity of thrombomodulin [52], suppresses thrombomodulin [51] and anticoagulant heparin sulfate expression [53], and blocks tissue plasminogen activator binding to human endothelial cells [54]. It also activates Factor XII [55], induces endothelial barrier dysfunction [56] and inhibits von Willebrand Factor processing and secretion [57]. Several studies have demonstrated the direct cytotoxic effects of homocysteine on endothelial cells grown in tissue culture [58–60]. It increases platelet adhesion and aggregation and inhibition of NA^+-K^+-ATPase activity and hemolysis of erythrocytes.

Mechanism of Hyperhomocysteinemia-Induced Vascular Dysfunction and Atherosclerosis

Hyperhomocysteinemia and Vascular Dysfunction

Impaired vasomotor regulation in monkeys with diet-induced moderate hyperhomocysteinemia has been reported [61]. The magnitude of vascular dysfunction was similar to that observed in atherosclerotic monkeys [62,63]. Vascular function normalizes within one month of decreasing plasma homocysteine concentration in non-atherosclerotic monkeys [61]. Lenz et al. [64] showed that atherogenic diet in monkeys produced both hypercholesterolemia and moderate hyperhomocysteinemia. They also showed that normalization of plasma homocysteine is insufficient to restore vascular function in atherosclerotic monkeys with persistent hypercholesterolemia. Atherosclerotic monkeys showed impaired carotid artery responses to nitroprusside, an endothelium-independent nitrovasodilator. Their observation that carotid artery responses to low doses of nitroprusside, and to a lesser extent, acetylcholine, improved after supplementation of vitamin B_6, B_{12}, and folic acid suggests that hyperhomocysteinemia may inactivate nitric oxide derived from exogenous and endogenous sources, through oxidative mechanism [65]. Impaired endothelium-dependent vasodilation has been shown to be associated with hyperhomocysteinemia by other investigators also [66].

Hyperhomocysteinemia and Atherosclerosis

Endothelial cell dysfunction/damage is prerequisite for development of atherosclerosis according to the injury hypothesis of atherosclerosis [67]. Homocysteine is toxic to the endothelial cells [58,59,68–71]. The endothelial cell injury could be due to generation of oxygen radicals produced by homocysteine. Oxygen radicals are known to mediate endothelial cell injury [72–74]. Sulfhydryl group of homocysteine is believed to act catalytically with ferric or cupric ions in a mixed function oxidation system to generate hydrogen peroxide, oxygen radicals and homocysteine radicals [75–77]. Reduced homocystine in the presence of copper ions in the cell culture medium is directly toxic to the cells, possibly due to oxygen radicals formed by thiol auto-oxidation [68]. Homocysteine is rapidly auto-oxidized in plasma to form homocysteine, mixed disulfides, and homocysteine thiolactate [78–80]. Superoxide anion and hydrogen peroxide are produced during auto-oxidation of homocyst(e)ine, and hydrogen peroxide (along with hydroxyl radical), in particular has been implicated in vascular injury of hyperhomocyst(e)inemia [77]. Homocysteine-induced endothelial cell injury in vitro is largely due to generation of hydrogen peroxide [70,78]. Auto-oxidation of homocysteine produces other cytotoxic oxygen radicals including superoxide anion and hydroxyl radical [81,82]. Homocysteine auto-oxidation has been shown to support the oxidation of low-density lipoprotein through generation of the superoxide anion radical [83]. The fact that toxic effect of homocysteine alone, and homocysteine plus Cu^{2+} was associated with increase in lipid peroxidation which was prevented by catalase and reduced by desferal [59], suggests that oxygen radicals are produced by homocysteine. Homocysteine may cause vascular injury by promoting the oxidation of low-density lipoprotein (LDL) [84]. Several studies have reported that sulfur-containing amino acids such as homocysteine, in the presence of a transition metal (iron or copper), cause oxidation of LDL [83–85]. Endothelial damage mediated through H_2O_2 production has been proposed [58,68,70,85].

It is not only that homocysteine may produce oxygen radicals but it may also reduce the antioxidant status which could injure endothelial cells. There is a decrease in the activity of antioxidant enzymes [superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-P_X)] of plasma of methionine-induced atherosclerosis in rabbits [87]. However, antioxidant enzymes of aorta increased with such treatment. In erythrocytes, activity of SOD increased, catalase remained normal and GSH-P_X decreased. The lipid peroxidation product malondialdehyde (MDA) increased both in plasma and aorta [87]. High concentrations of homocysteine increase intracellular reduced homocysteine which participates in the transsulfuration pathway and can replace cysteine in the synthesis of glutathione [78]. Homocysteine decreases intracellular glutathione and NAD^+ [58] and the ratio between intracellular concentration of reduced and oxidized glutathione [77]. Oxidized LDL [88] and oxygen radicals [89–92] have been implicated in the development of hypercholesterolemic atherosclerosis.

Homocysteine may also induce atherosclerosis by affecting endothelial-derived relaxing factor, nitric oxide (NO). NO combines with homocysteine in the presence of oxygen to form S-nitroso-homocysteine [76]. Nitrosation of sulfhydryl group of homocysteine inhibits sulfhydryl-dependent generation of hydrogen peroxide [76]. S-nitroso-homocysteine is a potent antiplatelet agent and vasodilator [93]. The protective effects of NO is compromised when homocysteine damages the endothelium to limit NO production. According to this model, vascular injury is caused by imbalance between NO production from dysfunctional endothelium and the levels of homocysteine [94]. Homocysteine may also decrease the bioavailability of NO by impairing its synthesis [65]. Homocysteine promotes lipid peroxidation which may decrease the expression of endothelial NO synthase and directly degrade NO [95–97]. Homocysteine suppresses the expression of cellular glutathione peroxidase by endothelial cells which may promote lipid peroxidation by oxygen radicals generated by homocysteine [98].

Homocysteine thiolactate, a byproduct of oxidation of homocysteine combines with LDL to form foam cells [99]. Homocysteine thiolactate has been suggested to facilitate the conversion of mitochondrial thioretinaco ozonide to thioco, and impairment of oxidative phosphorylation and enhancement of the proliferation and fiberosis of smooth muscle cells [100]. Homocysteine induced disturbance in oxidative metabolism leads to overproduction of oxygen radicals that induce endothelial injury [101]. Homocysteine also enhances the expression of monocyte chemoattractant protein-1 (MCP-1) in human aortic endothelial cells [102] which would help in development of atherosclerosis by transmigration of monocyte into subendothelial area.

Homocysteine could also take part in the development of atherosclerosis by smooth muscle cell proliferation. Homocysteine stimulates proliferation of vascular smooth muscle cells in culture [103]. Homocysteine may induce atherosclerosis by increased thrombogenicity [104] and induction of alterations in arterial connective tissue metabolism [105].

Homocysteine and Experimental Atherosclerosis

Experimental studies on the effects of homocysteine and methionine, a donor of homocysteine in the development of atherosclerosis, have been carried out in rabbits [87,106–110], rats [111–113], pigs [114], rhesus monkeys [115], and baboons [60,86]. Experimental evidence suggests that the severity of atherogenesis associated with homocysteinemia results from endothelial dysfunction and injury. Oral methionine administration to rats resulted in an increased number of endothelial cells in blood indicating injury to endothelium [106,109]. Fau et al. [111] observed disturbances in arterial wall morphology in rats chronically fed a diet enriched with 2% methionine. Atherosclerotic changes in methionine-fed rabbits also have been reported [106]. Matthias et al. [113] have shown that high doses of methionine increase the serum homocysteine in normotensive (NR) and spontaneously hypertensive (SHR) rats. Hyperhomocysteinemia was associated with considerable loss of endothelium and degeneration of aortic media, and elevated homocysteine and cystathione in aortic wall. Serum homocysteine and cystathione concentrations were higher in SHR and NR and methionine related aortic lesions were more pronounced and developed earlier in SHR than in NR. Chronic hyperhomocysteinemia produced in baboons by continuous intravenous homocysteine infusion results in patchy endothelial cell injury and focal proliferative intimal smooth muscle cell lesions similar in apearance to early atherosclerotic lesions in man [60,86]. Dipyridamole, an inhibitor of platelet function markedly reduced the intimal lesion formation with altering the amount of injured endothelium [60]. Sulfinpyrazone, an inhibitor of platelet function reduced the size and frequency of homocysteine-induced intimal lesions in baboons [86]. High methionine diet (3% D, L-methionine) increased plasma concentrations of triglycerides, cholesterol, homocysteine, cysteine, and lipid peroxides which were associated with development of atherosclerosis in rabbits [116]. Rabbits on 0.3% methionine diet for 6 to 9 months had increased plasma and aortic lipid peroxidation product, malondialdehyde (MDA) levels and aortic antioxidant enzyme activity [87]. However plasma antioxidant activity decreased. In erythrocytes, SOD activity increased, catalase activity remained normal, and GSH-P_X activity decreased. These changes were associated with the development of atherosclerosis in aorta. These results suggest that methionine-induced atherosclerosis may be associated with oxidative stress.

Homocysteine and Coronary Artery Disease

Several studies have shown high plasma homocysteine levels to be an independent risk factor for cardiovascular disease [11,12,33]. The first report, that patients with coronary artery disease frequently experienced abnormal homocysteine metabolism, was published in 1976 [117]. Until 1990, there were few reports on the role of homocysteine and coronary artery disease [11,19,118]. Since 1990, there has been an explosion in publications related to homocysteine and coronary artery disease [12,33,119–124]. Epidemiologic evidence from more than 20 case-control studies in-

volving 2000 patients has shown that elevated plasma homocysteine concentrations are associated with atherosclerosis [11,125]. In Physicians' Health Study, 14916, male physicians without known atherosclerosis had an initial homocysteine measurement done and were prospectively followed for an average of five years [121]. Men with plasma homocysteine concentrations 12% above the upper limit of normal had approximately a three-fold increase in the risk of myocardial infarction as compared with those with lower levels even after correction with other conventional risk factors. The prospective Trømso study [126] and other prospective studies [127] reported similar results. In the Trømso population based prospective study 21826 patients were involved. This study showed that baseline homocysteine levels were associated with the development of myocardial infarction and death during the follow-up period and that there was a graded risk of these adverse events throughout the normal range of homocysteine levels. There is one negative prospective study on myocardial infarction and stroke in relation to homocysteine from Finland [128]. This created some scepticism. However further study substantiated the earlier findings and mitigated the concern. Boushey et al. [12], based on the review of 27 studies which included 4000 patients, came to the conclusion that homocysteine is an independent risk factor for atherosclerotic disease in the coronary, cerebral and peripheral vessels. Using meta-analysis they estimated that 10% of the risk of coronary artery disease in the general population is attributable to homocysteine and that an increase of 5 μmoles/L in plasma homocysteine concentration raises the risk of coronary artery disease by as much as an increase in 20 mg per deciliter (0.52 μmoles/L) in cholesterol concentration.

In a prospective study involving 587 patients with angiographically documented coronary artery disease, initial homocysteine measurements were made and the patients were followed for a median of 4.6 years [129]. They found a strong graded association between plasma homocysteine concentration and overall mortality. There was a strong relationship between homocysteine concentrations and mortality above the homocysteine concentration of 15 μmoles/L. The adjusted mortality ratio was 1.6 above 15 μmoles/L of plasma homocysteine concentration as compared to those with values of 10 μmoles/L. This study showed a weak correlation between extent of coronary artery disease and total plasma concentration of homocysteine. Modest increase in the concentration of homocysteine (>15 to 20 μmoles/L) has been reported in patients with coronary artery disease, stroke, and peripheral vascular disease [12,33].

Stroke, Peripheral Vascular Disease and Homocysteinemia

Homocysteine concentration is elevated in patients with stroke and peripheral vascular disease [12,33,130]. The prevalence of carotid artery stenosis increases with increasing plasma concentrations of homocysteine [3]. In a cross-sectional study of 1041 elderly subjects, Selhub et al. reported a strong association between elevated concentrations of homocysteine and occlusive vascular disease even after adjustment for other conventional coronary risk factors. There was a graded relationship between plasma homocysteine and risk of carotid stenosis. The risk of carotid artery stenosis was increased even at plasma concentrations between 11.4 and 14.3 μmoles/L. Malinow et al. [4] reported similar findings. Hyperhomocysteinemia is associated with increased risk of stroke [10,131]. A prospective study reported a graded risk for stroke in middle aged British men [10]. The meta-analysis based on 27 studies involving 4000 patients showed that homocysteine was an independent risk factor for atherosclerosis in cerebral and peripheral vessels [12]. A population based cross-sectional study involving 2484 subjects showed that the magnitude of association between hyperhomocysteinemia and cardiovascular disease is similar for peripheral arterial, coronary artery and cerebrovascular disease in a 50 to 75-year old general population [132]. High plasma homocysteine may be a stronger (1.6 fold) risk factor for cardiovascular disease in subjects with non-insulin-dependent diabetes mellitus than in non-diabetic subjects. In the Rotterdam study, Bots et al. [133] showed that elevated homocysteine is associated with an increased risk of atherosclerosis and cardiovascular disease in subjects aged 55–74 years. There was no appreciable association of homocysteine levels to atherosclerosis and cardiovascular disease in subjects aged 75 and older. A single prospective study of homocysteine and vascular disease found no association between homocysteine concentrations and stroke [128]. In this population based study from Finland where the dietary consumption of folic acid is high, homocysteine concentrations were much lower than in the other studies and so was the frequency of vascular events.

Lower limb atherosclerotic disease is associated with high fasting homocysteine concentration [134]. There is increased concentration of homocysteine in patients with premature coronary artery, peripheral vascular and cerebrovascular disease [123,135,136]. High plasma homocysteine concentration and low concentrations of folate and vitamin B_6 are associated with increased risk of extracranial carotid artery stenosis in elderly [3,6]. Plasma homocysteine concentration is elevated in patients with carotid artery intimal wall thickening [4]. In normotensive subjects high normal homocysteine is associated with increased prevalence of carotid artery wall thickening [137]. Most studies have suggested that hyperhomocysteinemia is approximately as important as a risk factor for vascular disease as hypercholesterolemia, although homocysteinemia can be far more easily treated than hypercholesterolemia with inexpensive, non-toxic vitamin therapy [12]. Boers et al. [138] measured plasma homocysteine after methionine loading in 75 patients with premature atherosclerotic vascular disease. They observed that nearly 1/3 of all patients with cerebrovascular disease and peripheral vascular disease had hyperhomocystemia. Clarke et al. [119] reported that measurement of homocysteine after methionine loading in a cohort of men with premature vascular disease and normal controls showed that 42% of patients with cerebrovascular disease, 28% of patients with peripheral vascular disease and 30% of patients with coronary artery disease had hyperhomocysteinemia.

Venous Thrombosis and Hyperhomocysteinemia

Hyperhomocysteinemia is a common risk factor for recurrent venous thrombosis [6]. Patients with homocysteinemia have most frequent thrombotic complications such as deep venous thrombosis [34]. Mild hyperhomocysteinemia is also an independent risk factor for venous thromboembolism [139]. They reported a marked increase in the risk of venous thrombosis at the highest plasma homocysteine concentrations. A plasma homocysteine concentration of more than 22 μmoles/L increased the matched odds ratio for deep venous thrombosis to 4.0. den Heijer et al. [140] reported that plasma homocysteine concentrations of patients (25%) with recurrent venous thrombosis were high during fasting as well as during methionine loading tests. The combination of hyperhomocysteinemia and factor V Leiden further increases the relative risk of venous thromboembolism up to 3.6-fold [141]. Increased plasma levels of homocysteine in patients with venous thrombosis has also been reported by other investigators [142,143].

Renal Disease and Hyperhomocysteinemia

Kidney plays a part in homocysteine metabolism [144]. The incidence of vascular disease is increased with continuous ambulatory peritoneal dialysis [145] and acute dialysis [146,147]. Patients with renal failure, end-stage renal disease and chronic uremia have hyperhomocysteinemia and are at risk for atherosclerosis [32,148,149].

Diabetes Mellitus and Homocysteine

Diabetic (Type II) subjects who had macrovascular disease had higher fasting and post-methionine loading homocysteine than nondiabetic control subjects who were free of cardiovascular disease [150,151]. Hoogeveen et al. [132] have shown that hyperhomocysteinemia appears to be a stronger (1.6-fold) risk factor for cardiovascular disease in subjects with non-insulin-dependent diabetes mellitus than in subjects with normal or impaired glucose tolerance. In diabetic patients with intact renal function, plasma homocysteine level is normal [151,152] or even low [153]. In contrast, in diabetic patients with proteinuria and macrovascular disease, homocysteine levels are elevated [150,151,152] which may contribute to accelerated atherosclerosis. Homocysteine levels in plasma are lower in Type I (insulin-dependent diabetes mellitus) diabetic patients as compared to normal subjects [153]. Accelerated atherosclerosis in such patients could be due to factor(s) other than homocysteine.

Organ Transplant and Homocysteine

Homocysteine concentration was elevated in 70% of the patients after cardiac transplant and remained elevated for 12 months [135]. Associated with an increase in homocysteine were a decrease in plasma concentrations of vitamin B_{12} and folic acid. Homocysteine concentration is also elevated in patients with kidney, lung and liver transplant. The vascular complications in transplant patients may be due to hyperhomocysteinemia which is atherogenic and thrombogenic.

Interaction of Homocysteine and Conventional Risk Factor

There are numerous conventional risk factors for cardiovascular diseases including hypercholesterolemia, hypertension, cigarette smoking, and diabetes. This section will highlight the interaction and association of hyperhomocysteinemia and other conventional risk factors. There are numerous studies [41,124,126,154] which suggest a correlation between homocysteine and total cholesterol, HDL-cholesterol and LDL-cholesterol. Hyperhomocysteinemia has been shown to be an independent risk factor for atherosclerosis [124] and carotid-medial thickness [155] in hyperlipidemic patients. Plasma homocysteine concentration is positively associated with blood pressure in healthy population [41,156,157]. The correlation between plasma homocysteine concentration and carotid wall thickness is stronger in hypertensive than in normotensive subjects [4]. Cigarette smoking is associated with hyperhomocysteinemia [41]. Plasma homocysteine is elevated in patients with vascular disease who smoke [159]. It is a consistent finding that the relationship between plasma homocysteine concentration and vascular disease is strong even after adjustment for smoking. The interaction between homocysteine and three other risk factors (cholesterol, smoking, and hypertension) were studied [160]. The conclusion of this study was that the risk for cardiovascular disease with homocysteine was similar to and independent of conventional factors. The interaction of hyperhomocysteinemia with hypertension and smoking was strong, and the combined effect was more than multiplicative in both sexes but more so in female.

Therapeutic Regimen for Homocysteinemia

Reduction of dietary methionine is the appropriate approach in lowering plasma homocysteine, however it is severely limiting and virtually impossible to comply with. The most effective and easy way of reducing plasma homocysteine concentration is the treatment with folic acid, vitamin B_{12} and vitamin B_6 because these vitamins are involved in the metabolism of homocysteine.

Folic acid decreases the plasma concentration of homocysteine in normal subjects, and in patients with vascular disease and chronic renal failure [12,33,149]. Chauveau et al. [149] used folic acid (10 mg/day) and vitamin B_6 (70 mg/day) for two 3-month periods in nondialyzed chronic renal failure patients and concluded that folic acid but not vitamin B_6 was effective in lowering plasma homocysteine concentration. Naurath et al. [161] used folic acid (1.1 mg/day), vitamin B_{12} (1 mg/day) and vitamin B_6 (5 mg/day) to normalize elevated homocysteine concentration. In patients with coronary artery disease, folic acid in the doses of 0.4, 1.0 or 5 mg daily for 3 months reduced the homocysteine concentration by 30% in all treatment groups but remained unchanged in the placebo group [162]. Several other studies have shown that 0.65–10 mg/day of folic acid alone or in

combination with vitamin B_{12} and/or B_6 reduce fasting and post-methionine loading homocysteine by 25–50% in healthy, in hyperhomocysteinemic subjects and in patients with vascular disease [163–166]. No difference was found for 2.5 versus 10 mg/day of folic acid in patients with myocardial infarction [167]. Treatment with folic acid alone reduces homocysteine by 40–50% within 6 weeks. The doses were graded with initial dose of >5 mg/daily, then 3 mg, 2 mg, and 1 mg daily. Doses as low as 0.65 mg/daily have been shown to be effective, however the recommended dose is 1–2 mg/daily [165]. Patients with renal failure require much higher doses [146]. Folic acid is also efficacious in patients with the autosomal recessive thermolabile variant 5, 10-methylenetetrahydrofolic acid reductase. Individuals with elevated plasma homocysteine are normalized by treatment with folic acid or vitamin B_{12} [4,11,121,127]. A threshold for increasing homocysteine appears when folate concentration is less than 12.5 nmoles/L and for vitamin B_{12} at 225 pmoles/L [154]. In the USA the accepted lower limit for normal fasting plasma folate is 6.8 nmoles/L while the World Health Organization's lower limit is 13.6 nmoles/L. Folic acid is considered nontoxic and well tolerated for chronic use in high doses [168] except in patients with untreated vitamin B_{12} deficiency where folic acid treatment alone may exacerbate the symptoms of vitamin B_{12} deficiency. For more detail please refer to other studies [23,135,169].

Vitamin B_{12} reduces the plasma homocysteine to a maximum of 10–15% [165,170]. A low plasma vitamin B_{12} concentration may prevent optimal folic acid response [163]. A 1 mg/daily dose of vitamin B_{12} is recommended. Vitamin B_6, in doses of up to 300 mg daily, does not lower fasting plasma homocysteine concentration in healthy subjects or patients with vascular disease [163,165,170]. However lower doses (10–250 mg/day) of vitamin B_6 reduces post-methionine loading hyperhomocysteinemia in most patients receiving folic acid [164,171]. Chronic use of vitamin B_6 may precipitate peripheral neuropathy but a daily dose of 100 mg or less is safe [172].

Comments

Numerous questions arise out of this review. Should all patients with atherosclerotic vascular disease be screened for homocysteinemia? It is good practice to have homocysteine measured in all cases of coronary artery and atherosclerotic disease. However, this will increase the health care cost. There are patients with coronary artery and other atherosclerotic disease in whom the other conventional risk factors (lipids) are normal. It would be advisable to measure the homocysteine levels in such patients considering homocysteine is an independent risk factor for cardiovascular disease. What if it is high? Since vitamin supplementation reduces the homocysteine level, it should be tried. Clinical trials should be started to determine if such therapy affects the outcome in such patients. While we are waiting for such trials, people, especially prone to cardiovascular disease, should use folic acid and vitamin B_{12}. The encouraging news is that in the USA, products made with cereal grain or flour will be fortified by the addition of 140 μg of folic acid per 100 g of flour. This is intended to reduce the incidence of neural defects, but it will also reduce homocysteine concentration and decrease the risk of atherosclerosis associated diseases in the general population.

Conclusion

Prospective and case-control studies have shown that hyperhomocysteinemia is associated with coronary artery disease, carotid stenosis, cerebrovascular disease and thromboembolic peripheral vascular disease. It is a strong independent risk factor and remains strong even after adjustment of other conventional risk factors such as lipids, hypertension, diabetes, and smoking. The evidence suggests that atherogenicity of homocysteine could be due to oxidative stress, thromboembolism, and vascular smooth muscle proliferation. Interaction of hyperhomocysteinemia with cholesterol, hypertension and smoking is strong, and the combined effect is additive with cholesterol, and more than multiplicative with hypertension and smoking. Patients with elevated homocysteine should be treated with folic acid (1–2 mg/day). It is safe and efficient. In addition to folic acid, vitamin B_{12} and vitamin B_6 should be used.

Summary

Homocysteine has emerged as a risk factor for atherosclerotic cardiovascular disease. It is a sulphur-containing amino acid derived from methionine. Its metabolism involves remethylation, transsulfuration and demethylation processes which requires folic acid, vitamin B_{12} and vitamin B_6. The normal value of homocysteine is between 5 and 15 μmoles/L. Hyperhomocysteinemia (>15 μmoles/L) is caused by numerous factors including deficiency in folic acid and vitamins B_6 and B_{12}, enzyme (cystathione β-synthase, methionine synthase, methylenetetrahydrofolate reductase) deficiency and certain drugs (methotrexate, phenytoin, nitrous oxide, theophylline, L-dopa, cholestyramine, niacin), smoking, and chronic alcohol and coffee consumption. Homocysteine produces atherosclerosis, vascular endothelial damage, vascular smooth muscle cell proliferation and thromboembolism. Thromboembolism is due to activation of factor V and XII, inhibition of von Willebrand factor processing and secretion, reduction of protein C activation and inactivation and suppression of thrombomodulin. There is strong evidence that endothelial cell injury is due to increased production of oxygen radicals and decreased level of antioxidants. Homocysteine-induced atherosclerosis is suggested to be due to various factors including, endothelial cell damage, smooth muscle cell proliferation, thromboembolism, and inability to sustain S-nitroso-homocysteine (a potent vasodilator and inhibitor of platelet aggregation) formation because of an imbalance between nitric oxide production by dysfunctional endothelium and homocysteine.

Population based prospective and cross sectional studies suggest that homocysteine is an independent and strong risk factor for atherosclerotic disease in coronary, cerebral, and peripheral vessels. There is a strong relationship between homocysteine concentration and mortality in patients with

coronary artery disease. Homocystein levels are elevated in patients with stroke, carotid artery stenosis, and other peripheral vascular diseases, diabetes, hypertension, renal disease, and organ transplant. Numerous studies suggest a correlation between homocysteine and total HDL- and LDL-cholesterol. Homocysteine is an independent and strong risk factor for atherosclerosis and carotid medial thickness in hyperlipidemic patients. It is positively associated with blood pressure in healthy populations. The interaction of hyperhomocysteinemia with hypertension and smoking is strong and the combined effect is more than multiplicative. Vitamin (folic acid, vitamin B_{12}, and B_6) supplementation reduces plasma homocysteine levels. There is no unanimity in the doses of these vitamins in reduction of homocysteine. However, recommended doses of folic acid, vitamin B_{12}, and vitamin B_6 are 1 mg, 1 mg, and 5 mg respectively. Folic acid is nontoxic. Its use should be combined with vitamin B_{12} to ward off exacerbation of symptoms of vitamin B_{12} deficiency. Chronic use of vitamin B_6 may precipitate peripheral neuropathy but a dose of 100 mg or less daily is safer.

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