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Homocysteine- The Hidden Factor and Cardiovascular Disease: Cause or Effect?

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Abstract

Markedly or mildly elevated circulating homocysteine concentrations are associated with increased risk of vascular occlusion. Here we review possible mechanisms that mediate these effects. Inborn errors of homocysteine metabolism result in markedly elevated plasma homocysteine (200-300 μmol/L) and thromboembolic (mainly venous) disease which is easily normalized with oral folate and ongoing trials are assessing the effect of folate treatment on outcomes. Some people have a common genetic variant (called methylenetetrahydrofolate reductase, abbreviated *MTHFR*) that also impairs their ability to process folate. Indeed, there are evidences suggesting an acute antioxidant effect of folic acid on homocysteine concentrations. This antioxidant mechanism may oppose an oxidant effect of homocysteine and be relevant to treatment of patients with vascular disease, especially those with chronic renal insufficiency. Such patients have moderately elevated plasma homocysteine and greatly increased cardiovascular risk that is largely unexplained.

Keywords: Metabolic disorders; Homocysteine; Renal dysfunction; Methylenetetrahydrofolate reductase; folic acid

Introduction

Homocysteine also known as the H-factor, is a naturally occurring amino acid and is a by-product of methionine metabolism in the body. It is a common amino acid (one of the building blocks that make up proteins) found in the blood and is acquired mostly from eating meat. In 1969, a connection between homocysteine (a sulfur-containing amino acid) and cardiovascular disease was proposed when it was observed that people with a rare hereditary condition called homocystinuria are prone to develop severe cardiovascular disease in their teens and twenties. In this condition, an enzyme deficiency causes homocysteine to accumulate in the blood and to be excreted in the urine. Abnormal homocysteine elevation also occurs among people whose diet contains inadequate amounts of folic acid, vitamin B6, or vitamin B12. Regardless of the cause of the elevation, supplementation with one or more of these vitamins can lower plasma homocysteine levels.

Studies done in the 1980s and 1990s linked elevated blood levels of homocysteine to increased risk of premature coronary artery disease, stroke, and venous blood clots, even among people with normal cholesterol levels [1,2]. These studies led to speculations that high homocysteine levels could contribute to atherosclerosis in at least three ways: (a) a direct toxic effect that damages the cells lining the inside of the arteries, (b) interference with clotting factors, and (c) oxidation of low-density lipoproteins (LDL).

Homocysteine is a hidden toxic chemical that is not meant to accumulate unchecked in the body. It needs to be transformed into safer amino acids, like methionine and cysteine. Homocysteine levels increases in the plasma due to some metabolic problems that can be inherited or can result from nutritional deficiencies and is known to be a major risk factor for atherosclerosis, coronary heart disease, stroke and Alzheimer's disease [3,4]. Blood levels of homocysteine tend to be highest in people who eat a lot of animal protein and consume few fruits and leafy vegetables, which provide the folic acid and other B vitamins that help the body rid itself of homocysteine [5]. Homocysteine pathways normally lead to the production of other essentials including glutathione a powerful detoxifier and various hormones like serotonin (the happy hormone), melatonin (sleep and mood improving hormone), dopamine (euphoria hormone) and adrenaline (the fight and flight hormone).

Homocysteine is present in plasma in four different forms: around 1% circulates as free thiol, 70-80% remains disulphide-bound to plasma proteins, mainly albumin and 20-30% combines with itself to form the dimer homocysteine or with other thiols [6]. Homocysteine is a key determinant of the methylation cycle [7]. It is methylated to methionine, which undergoes S-adenosylation and forms S-adenosylmethionine (SAM) [7]. S- adenosylmethionine is the principal methyl donor for all methylation reactions in cells [7]. Condensation of methionine with ATP, leads to the formation of SAM (S- Adenosylmethionine) [8]. The methyl group attached to the tertiary sulphur of SAM can be transferred and therefore can cause methylation of other substances. This methylation is accompanied by energy loss, so this reaction is irreversible. The demethyation reaction leads to the formation of SAH (S- adenosylhomocysteine) [8]. SAH is a thioether (a sulfur bonded to two alkyl or aryl groups) analogous to methionine. The SAM-to-SAH ratio defines the methylation potential of a cell [7]. Hydrolysis of SAH leads to the formation of homocysteine and adenosine [8]. This homocysteine can be used in one of two ways:

a) In case of methionine deficiency, homocysteine can be re-methylated to form methionine [8]. The enzyme N5, N10-methylenetetrahydrofolate reductase converts homocysteine to methionine [9].

b) In presence of sufficient methionine, homocysteine is instead used to produce cysteine [8]. Cystathionine-β-synthase is an enzyme (with pyridoxine or vitamin B_6 as an essential cofactor) that converts homocysteine to cysteine [8]. Homocysteine is synthesized from the essential amino acid methionine, therefore cysteine is not an essential amino acid as long as sufficient methionine is available [8] (Figure 1).

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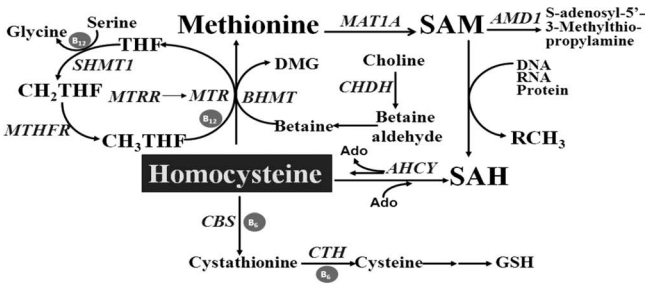
Biochemical Basis of Hyperhomocysteinemia

Hyperhomocysteinemia is defined as a medical condition characterized by an abnormally high level (>15 µmol/L) of homocysteine in the blood [10]. Total concentration of homocysteine in plasma of healthy humans (fasting) is low and its level is between 5.0 and 15.0 µmol/L when assessed with the use of HPLC, or 5.0-12.0 µmol/l when immunoassay methods are used [11]. When the level is between 16-30 µmol/L it is classified as moderate, 31-100 µmol/L is considered intermediate and a value above 100 µmol/L is classified as severe hyperhomocysteinemia [6]. There are two types of hyperhomocysteinemia: (a) the rare but severe forms are due to major genetic mutations of the enzymes implicated in homocysteine metabolism; (b) the more common forms cause moderately elevated homocysteine levels related to a pathogenesis such as genetic and environmental factors [9].

Three rare autosomal recessive disorders (the homocystinurias), in which homozygotes lack one of the three principal enzymes in homocysteine metabolism, cause extremely high serum homocysteine levels (> 100 ìmol/l) and a high risk of premature cardiovascular disease (affecting half of homozygotes by the age of 30) [7]. The biochemical change that is common to all three disorders is a high serum homocysteine concentration (no other substance is consist ently high or low) [8,9], indicating that high homocysteine levels cause cardiovascular disease. Whether moderate increases in serum homocysteine cause cardiovascular disease has been the subject of debate. Moderate increases occur as a result of a mutation in the gene coding for the enzyme methylenetetrahydrofolate reductase (MTHFR) in which cytosine is replaced by thymidine (C-T) at base position 677 of the gene. This variant of the enzyme has reduced activity [10], resulting in an elevation of serum homocysteine concentrations of about 20%.



AHCY	S-Adenosylhomocysteine hydrolase	*CTH*	Cystathionine gamma-lyase
AMD1	Adenosylmethionine decarboxylase 1	*MAT1A*	Methionine adenosyltransferase I, alpha
BHMT	Betaine-homocysteine methyltransferase	*SHMT1*	Serine hydroxyl methyltransferase 1
CBS	Cystathionine-beta-synthase	*CHDH*	Choline dehydrogenase
MTR	5-Methyltetrahydrofolate-homocysteine methyltransferase		
MTRR	5-Methyltetrahydrofolate-homocysteine methyltransferase reductase		
MTHFR	5,10-methylenetetrahydrofolate reductase		

Figure 1: Homocysteine metabolism.

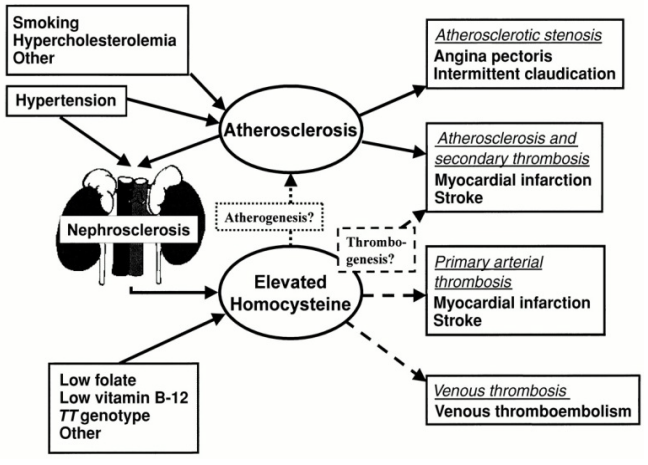


Figure 2: Various metabolic disorders related to elevated homocysteine level in humans.

Homocysteine and Metabolic Disorders

Severe hyperhomocysteinemia (HHC), also known as homocystinuria, is a rare autosomal recessive disorder caused by Cystathionine beta synthase (CBS) deficiency and is an inherited disorder of the metabolism of the amino acid methionine. This defect leads to a multisystemic disorder of the connective tissue, muscles, central nervous system, and cardiovascular system [11] (Figure 2).

Hyperhomocysteinemia has received increasing attention during the past decade and has joined smoking, dyslipidemia, hypertension, and obesity as an independent risk factor for cardiovascular disease. In addition to its role in cardiovascular disease, increased homocysteine levels have been implicated in a variety of other clinical conditions, including neural tube defects, spontaneous abortions, placental abruption, low molecular weight, renal failure, non-insulin dependent diabetes, rheumatoid arthritis, alcoholism, osteoporosis and neuropsychiatric disorders.

Analyses of homocysteine usually involve fasting samples of either serum or plasma. The concentrations are higher in serum, and an increase of 10% have been reported in the postprandial stage [9]. Homocysteine levels also increases with age and are higher in men than in women. A variety of diseases and medications modify homocysteine concentration, and notably, impaired renal function may greatly increase homocysteine levels [8]. Measurement of homocysteine should avoid blood samples that have been stored at room temperature, because red blood cells may release homocysteine, causing an artifactual increase in extracellular homocysteine concentrations.

A complicating aspect of homocysteine metabolism for cardiovascular studies is that homocysteine concentrations may increase after a myocardial infarction or a stroke. Critically, data are not available for samples obtained before and after an event. However, analysis of samples obtained at the time of a myocardial infarction and up to 180 days later indicated an increase in homocysteine concentration from 13 to 16 mmol/L [6]. Similarly, samples collected within 2 days of a stroke and up to 645 days later exhibited a rise in homocysteine concentration from 11.4 to 14.5 mmol/L [7]. *In vitro*, a very wide range of effects have been attributed to homocysteine [4]. These include direct damage to endothelial cells, flawed platelet activity, elevated procoagulant activity, increased collagen synthesis, and enhanced proliferation of smooth muscle cells, all these are related to the pathogenesis of cardiovascular disease.

Homocysteinuria and Thrombosis/Atherosclerosis

Various studies have shown that mild hyperhomocysteinemia is a risk factor for venous thromboembolic disease and seems to be associated primarily with thrombotic disease, which predominantly affects veins [12,13]. It has been proposed that Hcy-caused endothelial injury may be mediated by oxidative stress, attenuation of NO-mediated vasodilatation, and disturbance in the antithrombotic activities of the endothelium [9]. During the early stages of atherosclerosis, stimulation of endothelial cells results in the secretion of various chemokines and adhesion molecules, leading to the recruitment of leukocytes including

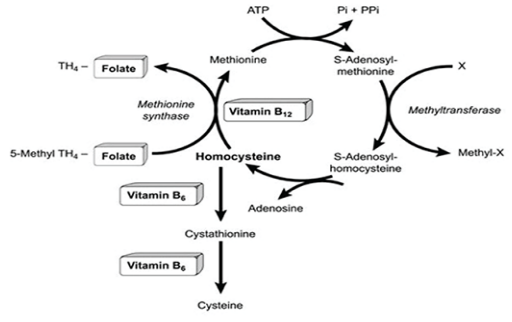
monocytes to the vascular wall [10]. It has been reported that the expression of monocyte chemoattractant protein-1 (MCP-1), a potent chemokine for monocytes, was found to be markedly elevated in Hcy-treated endothelial cells, vascular smooth muscle cells (VSMCs), and macrophages. The activation of a transcription factor, nuclear factor kappa-B (NF-kβ), has been linked to the expression of inflammatory factors during the onset of atherosclerosis [14-16]. Hcy treatment caused an activation of NF-kβ, leading to increased chemokine expression in VSMCs and macrophages [12,13]. NF-kβ is normally present in the cytoplasm in an inactive form that is associated with an inhibitory protein named IkBα.[15] In the presence of various NF-kβ stimuli, IkBα (a well-studied IkBα protein) is rapidly phosphorylated, leading to ubiquitination and subsequent degradation of IkBα as well as translocation of NF-kβ into the nucleus. Phosphorylation of IkBα is mediated by enzymes called IkBα kinases (IKKs) [16]. After dissociation from IkBα, the active NF-kβ is translocated into the nucleus, where it binds to the IkBα -binding motifs in the promoters or enhancers of the genes encoding cytokines [17-19]. It was first time demonstrated that Hcy-induced expression of inducible NO synthase in VSMCs was mediated via NF-kβ activation [20-22]. In contrast, little or no activation of NF-kβ is found in normal aorta or arteries. The expression of endothelial MCP-1 and adhesion molecules was significantly elevated *in vitro* and *in vivo* [23]. In rats with diet induced hyperhomocysteinemia, the expressions of MCP-1, vascular cell adhesion molecule-1 (VCAM-1), and E-selectin in the aortic endothelium were significantly increased, leading to enhanced monocyte binding to the endothelium [22]. It was hypothesize that the activation of NF-kβ might play an important role in the expression of these inflammatory factors induced by Hcy in endothelial cells. Taken together, results obtained from *in vivo* and *in vitro* experiments suggest that Hcy-induced NF-kβ activation in endothelial cells may represent one important mechanism by which Hcy causes atherosclerosis [23]. Multiple pathways mediating NF-kβ activation have been proposed. Reactive oxygen species have been implicated to stimulate IkBα degradation and NF-kβ activation in vascular cells [23]. It was demonstrated that oxidative stress contributed to vascular dysfunction in animal models with hypercholesterolemia- induced atherosclerosis [23]. Superoxide levels were significantly increased in the vascular wall, causing impaired vessel relaxation in hypercholesterolemic rabbits [23]. It was reported that hydrogen peroxide stimulated NF-kβ activity via activation of IKK in HeLa cells [21].

In experimental models, Hcy administration has caused endothelial cell injury, both *in vitro* and *in vivo*. Hcy induces oxidative stress to the endothelium and reduces available nitric oxide. Hcy may also generate free radicals and inhibit the production of other antioxidants [23]. Endothelial injury is followed by platelet aggregation and thrombus formation. Toxic endothelial damage is also related to the stimulation of smooth muscle cell proliferation and susceptibility to oxidation of low density lipoproteins. Another mechanism by which Hcy can induce vascular injury is the increased thrombogenicity mediated by increased platelet adherence and the release of platelet derived growth factors, activated Factor V, X, and XII, inhibition of protein C activation, inhibition of cell surface expression of thrombomodulin, and decreased tissue plasminogen activator activity [21]. Hcy has also been known to increase arterial stiffness by damaging elastin fibers, increasing collagen production and stimulating smooth muscle activity [3]. Antioxidants were shown to be able to block IkBα degradation and NF-kβ activation. Oxidative stress has been proposed to be an important mechanism of Hcy-induced endothelial dysfunction. Administration of antioxidants such as vitamins were shown to alleviate the adverse effect of Hcy [12].

Mechanism of Homocysteine Contribution to Vascular Injury

Several studies, some population-based, have linked plasma homocysteine levels to blood pressure, especially systolic pressure. In addition, the association may be confounded by renal function. Observations that homocysteine-lowering therapies with folic acid-based treatments have been followed by decreases in blood pressure, however, raise the possibility that the link between homocysteine and blood pressure is real, which is important as, homocysteine levels can easily be lowered by folic acid-based regimens [24]. Mechanisms that could explain the relationship between homocysteine and blood pressure include increased arterial stiffness, endothelial dysfunction with decreased availability of nitric oxide, low folate status, and insulin resistance. So far, however, no evidence has been provided that these mechanisms are operative in humans. Some studies have shown that vitamin B_6, B_{12} and folic acid have proved to be beneficial in hyperhomocystenuria [25-27]. The mechanism of action of these vitamins is shown in Figure 3. Ongoing large intervention studies with homocysteine-lowering vitamins may indicate whether blood pressure is indeed lowered by these vitamins, whether the blood pressure decrease, if any, is explained by the decrease in homocysteine levels, and whether a vitamin treatment-associated decrease in cardiovascular morbidity (if any) is explained by the decrease in blood pressure. The vascular risk associated with hyperhomocysteinaemia has been observed to be stronger in hypertensive individuals. More recently, attention has been focussed on the direct relations of plasma homocysteine to blood pressure and hypertension because of the suggestion that the adverse risk associated with hyperhomocysteinaemia might be mediated in part by the positive association of homocysteine with hypertension [8]. Because homocysteine levels are more strongly associated with systolic than diastolic blood pressure, it can be hypothesized that hyperhomocystenemia enhances arterial stiffness. The main consequences of increased arterial stiffness are an increase in pulse pressure, mainly due to elevated systolic blood pressure and cardiac load [23]. Arterial stiffness is determined by the number and function of smooth muscle cells, by extracellular matrix properties such as quantity and collagen to elastin ratio and also by endothelial function.

Homocysteine might increase arterial stiffness by destroying elastin fibres, increasing collagen production and by stimulating smooth muscle cells (SMC) activity. Apart from increasing stiffness of large conduit arteries, SMC proliferation may also lead to high b.p. by increasing total peripheral resistance in arteries. *In vitro* and animal studies have shown that high Hcy concentration leads to SMC proliferation, increased collagen production and elastin fibre formation



S-adenosyl homocysteine is formed during S-adenosyl methionine-dependent methylation reactions, and the hydrolysis of S-adenosyl homocysteine results in homocysteine. Homocysteine may be remethylated to form methionine in a reaction that requires both folate and vitamin B_{12}. Alternately, homocysteine may be metabolized to the amino acid, cysteine, in reactions catalyzed by two vitamin B_6-dependent enzymes.

Figure 3: Involvement of B vitamins in homocysteine metabolism.

S. No.	Groups	Homocysteine		Systolic B.P		Diastolic B.P.	
		Mean ± SEM	SD	Mean ± SEM	SD	Mean ± SEM	SD
1	Tested	38.340 ± 2.411	15.2	160 ± 2.067	13.071	93.90 ± 1.04	6.5
2	Control	7.360 ± 0.224	1.00	124 ± 1.841	8.233	82.40 ± 1.404	6.2
3	Tested male	36.387 ± 2.748	15.0	158.2 ± 2.113	11.571	93.2 ± 1.285	7.0
4	Control male	7.5338 ± 0.346	1.1	123.00 ± 2.714	9.4	82.667 ± 1.831	6.3
5	Tested female	44.2 ± 4.766	10.0	165.8 ± 5.107	16.151	96 ± 1.461	4.6
6	Control female	7.10 ± 0.209	0.5	125.5 ± 2.26	6.392	82.00 ± 2.33	6.5
Note: Tested; group consists of both male and female in general having high Hcy conc.							

Table 1: Correlation between homocysteine and blood pressure.

[23]. Homocysteine might also decrease arterial stiffness by impairing collagen cross-linking. *In-vivo* study conducted on minipig model, diet induced hyperhomocysteinemia resulted in a "mega artery syndrome" with hyperpulsatile arteries, characterised by hypertension extended reactive hyperaemia of conduit arteries and dilatation of the aorta [23]. In these animals, a decreased aortic stiffness was observed in the presence of fragmentation of the arterial wall elastic lamina.

In Table 1, from a study conducted on human, we can see that with increasing Hcy concentration in tested groups (male female and general) leads to increase in the systolic and diastolic blood pressure as compared to the control groups. Also the elevation in systolic blood pressure is more as compared to the dystolic blood pressure. An almost normal dystolic blood pressure i.e. the bottom number of a blood pressure measurement ≤90 mm Hg and a systolic blood pressure; the top number of a blood pressure measurement, greater than 140 mm Hg is called isolated systolic hypertension. Isolated systolic hypertension can be caused by underlying conditions such as artery stiffness or heart valve problems. It is already discussed and is evident also from this data that elevated Hcy concentration leads to artery stiffness which may show a fluctuation in blood pressure.

Conclusion

In conclusion, severe elevation of homocysteine concentration in patients with homocystinuria leads to a high incidence of premature atherothrombotic events. *In vitro* and *in vivo* studies demonstrate a plethora of biologically plausible mechanisms that implicate homocysteine in promoting atherosclerotic and thrombotic vascular disease. The association of homocysteine with cardiovascular disorders has been studied extensively since the relationship was first proposed in 1969. From the wealth of data available, it seems reasonable to assume that moderately elevated homocysteine is an independent risk factor for cerebrovascular, peripheral vascular, coronary heart and venous thromboembolism disease. Taking a multivitamin that contains approx. 400 micrograms of folic acid in addition to what we might get from our diet is highly recommended to homocystenuria patients. Physical exercise and reducing foods high in animal protein can also help to lower homocysteine levels.

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