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Homocysteine— To Test and to Treat

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Homocysteine is receiving a lot of attention these days as a new risk factor for a variety of diseases. During the past 10 years, the vast majority of over 100 case-control retrospective studies have shown that homocysteine is a strong independent risk factor for coronary artery disease, cerebrovascular disease and peripheral vascular disease.¹ Prospective studies also suggest that homocysteine is a risk factor for cardiovascular disease, but not all of the prospective studies have been positive.² Recent studies show that hyperhomocysteinemia is of particular concern in patients with preexisting conditions such as coronary artery disease, renal failure, peripheral arterial disease, diabetes and venous thromboembolism. Higher elevations of homocysteine in these patients affect short-term outcomes with respect to mortality and vascular events.³ Homocysteine is an emerging risk factor for cognitive dysfunction disorders such as vascular dementia and Alzheimer's disease.⁴

What is homocysteine, where does it come from, and how is it metabolized?

Homocysteine is a normal metabolite of the essential amino acid methionine (Figure 1). Structurally, it closely resembles methionine and cysteine; all three amino acids contain sulfur. They are metabolically linked to each other as shown in Figure 2. Since foods contain little or no homocysteine, nearly all of the homocysteine in the body is derived from the methionine in animal and plant proteins.

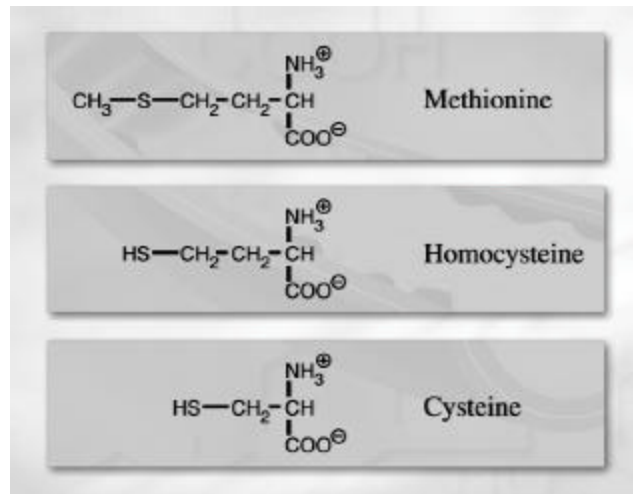


Figure 1. Structures of methionine, homocysteine and cysteine.

Homocysteine metabolism is driven by several B-complex cofactors.⁵ Folate and vitamins B₂, B₆ and B₁₂ are used in the remethylation pathway; vitamin B₆ is used in the transsulfuration pathway (Figure 2). Deficiencies of folate, vitamin B₆ or vitamin B₁₂ can lead to impaired homocysteine metabolism and hyperhomocysteinemia. In addition, mutations in the genes coding for methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MS) and cystathionine β-synthase (CBS) may also produce hyperhomocysteinemia. Subjects who inherit two identical defective alleles may have little or no enzyme

activity (e.g., for CBS). This can result in severe hyperhomocysteinemia and the rare disease known as homocystinuria. Without treatment, 50 percent of affected individuals will experience a thromboembolic event before age 30.⁶

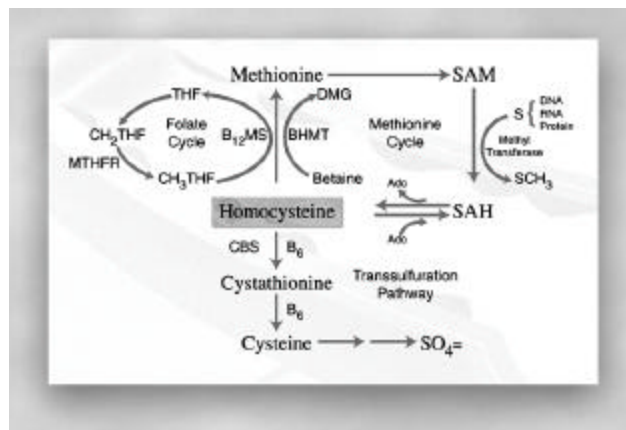


Figure 2. Major pathways of homocysteine metabolism in the liver and kidneys. Homocysteine is generated in a cycle through S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH). Remethylation of homocysteine back to methionine is carried out by vitamin B₁₂-dependent methionine synthase (B₁₂MS) and betaine-homocysteine methyltransferase (BHMT). Homocysteine is also converted to cysteine through the transsulfuration pathway, initiated by B₆-dependent cystathionine β-synthase (CBS). The folate cycle generates 5-methyltetrahydrofolate (CH₃THF) for the remethylation of homocysteine. Other abbreviations: DMG, dimethylglycine; Ado, adenosine; THF, tetrahydrofolate; CH₂THF, 5,10-methylenetetrahydrofolate; SO₄⁼, sulfate.

The determinants of mild hyperhomocysteinemia, commonly seen in patients with cardiovascular disease, are multifactorial and involve both genetic and acquired components. Gene-nutrient interactions such as homozygosity for thermolabile MTHFR and low-folate nutritional status can result in mild hyperhomocysteinemia. Approximately 12 percent of the Caucasian population is homozygous for thermolabile MTHFR. Smoking, excessive coffee consumption and lack of exercise are associated with elevations in homocysteine as well.^{7,8}

What is hyperhomocysteinemia and how is it determined?

The term can be defined simply as “elevated blood homocysteine” but the actual situation is more complex. When homocysteine is transported out of cells into circulation, it reacts with other compounds containing sulfhydryl (RSH) or disulfide (RS-SR) groups. As a result of these reactions almost all of the homocysteine in circulation is converted to a disulfide (oxidized) form. Less than 1 percent of total plasma homocysteine is found as the free RSH form. The disulfide forms include the symmetrical dimer homocystine and mixed disulfides with cysteine and plasma proteins containing free cysteine residues (Figure 3). In fact, over 80 percent of circulating

homocysteine is carried as a mixed disulfide by plasma proteins.⁹

Sensitive and reliable assays for plasma total homocysteine (tHcy) were developed in the mid- to late 1980s. This technical achievement was largely responsible for establishing homocysteine as a major independent risk factor for cardiovascular disease. In practice, plasma samples are treated with strong reducing agents to break disulfide bonds, thus liberating free homocysteine and other small thiols such as cysteine and glutathione. The thiols are usually derivatized with a reporter group, separated and detected. Thiol-specific fluorescent reporter groups are commonly used, and separations are achieved by high-performance liquid chromatography (HPLC), after which the compounds are detected fluorometrically (HPLC-FD). Other methods use HPLC with electrochemical detection (HPLC-ED), or gas chromatography with mass spectrometry (GC/MS). Immunoassays based on the conversion of homocysteine to S-adenosyl-homocysteine were introduced about five years ago and have become the mainstay of commercial assays.

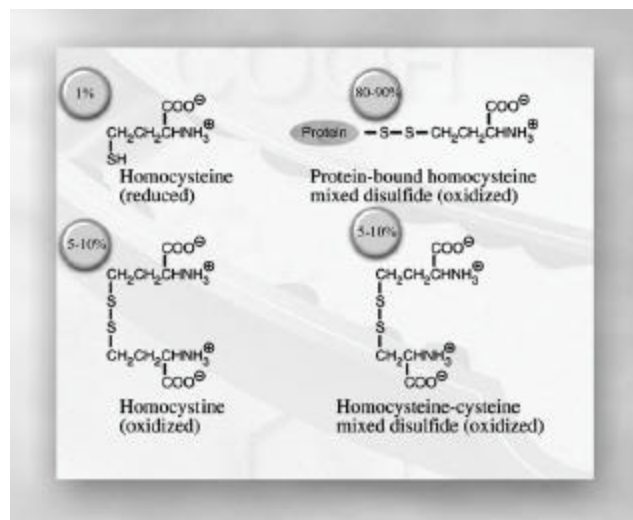


Figure 3. The circulating forms of homocysteine that make up plasma total homocysteine.

What is a “normal” plasma tHcy?

Until recently, the normal range for plasma tHcy was considered to be 5 to 15 $\mu\text{mol/L}$. It is now widely accepted that the upper range of normal may be 10 to 12 $\mu\text{mol/L}$ for middle-aged adults and that risk for cardiovascular disease occurs if plasma tHcy exceeds this value. However, it is now also recognized that homocysteine levels increase with age, perhaps as a result of micronutrient deficiencies due to malabsorption. In the future, it is likely that age-specific reference ranges will be established. Premenopausal women have approximately 20 percent lower values than their male counterparts, suggesting that homocysteine metabolism may be regulated to some extent by hormones.

Patients with coronary artery disease and other cardiovascular diseases usually have mild hyperhomocysteinemia (>12 to 25 $\mu\text{mol/L}$) with an incidence of 30 to 50 percent.¹⁰ Almost all patients with end-stage renal disease have hyperhomocysteinemia that tends to be of an intermediate form (>25 to 50 $\mu\text{mol/L}$).¹¹ Little or no homocysteine is excreted by the normal kidney. The role of the kidney in homocysteine metabolism and the regulation of homocysteine metabolism is poorly understood. The homocystinurias, those rare inborn errors of homocysteine metabolism, are associated with severe hyperhomocysteinemia (>50 to 500 $\mu\text{mol/L}$) and premature atherosclerosis and thrombosis.

How does homocysteine injure blood vessels?

Because homocysteine is a thiol, it can undergo autooxidation and oxidation with other thiols. The resulting reactive oxygen species—hydrogen peroxide and superoxide anion radical—generate oxidative stress. The concentration of plasma total cysteine is 20 to 30 times higher than that of plasma tHcy, yet cysteine, which also undergoes similar oxidative reactions, is not usually considered a risk factor.¹² If oxidative stress is not the mechanism for homocysteine-induced vascular dysfunction, is there perhaps another, more attractive hypothesis? Yes, and it is related to direct molecular targeting by homocysteine. Recent evidence suggests that homocysteine may limit the bioavailability of nitric oxide, resulting in the impairment of flow-mediated vasodilatation. The limited bioavailability of nitric oxide could be due to nitrosothiol formation with homocysteine. Homocysteine may also target specific proteins and impair their activity and function through disulfide bond formation. The decreased binding of tissue plasminogen activator to homocysteine-modified annexin II is a case in point and may explain, in part, the procoagulant activity of homocysteine.¹³ Finally, as shown in Figure 4, homocysteine appears to induce the expression and secretion of chemokines such as monocyte chemoattractant protein 1 (MCP-1) and interleukin 8 (IL-8) in vascular endothelial cells.¹⁴ Production of these chemokines by stimulated endothelial cells would attract monocytes and neutrophils to sites of vascular injury where they could take up residence in the intimal space.

Is hyperhomocysteinemia a treatable disease?

Once a diagnosis of hyperhomocysteinemia has been made, it is safe and easy to lower plasma tHcy in most individuals. A cocktail of folic acid (400 to 800 μg), vitamin B₁₂ (100 to 500 μg) and vitamin B₆ (25 to 100 mg) will reduce plasma tHcy by up to 40 percent in subjects with cardiovascular disease. Whether lowering homocysteine will have a beneficial effect on disease progression will be known in 3 to 5 years after the completion of a dozen or so worldwide clinical trials involving over 70,000 subjects.

Should everyone be tested for plasma tHcy?

The American Heart Association has recommended that individuals with a family history of heart and cardiovascular disease be tested for plasma tHcy. Other subjects who should be tested are those with premature atherosclerosis or atherosclerosis with no known conventional risk factors such as hypertension or hyperlipidemia. Hypercoaguable profiles now routinely include plasma tHcy. Of growing concern is the increased incidence of cognitive dysfunction disorders, such as vascular dementia and Alzheimer's disease, and the possibility that micronutrient deficiencies resulting in hyperhomocysteinemia play a causative role. It may be common practice in the near future to test everyone over the age of 60 for plasma tHcy.

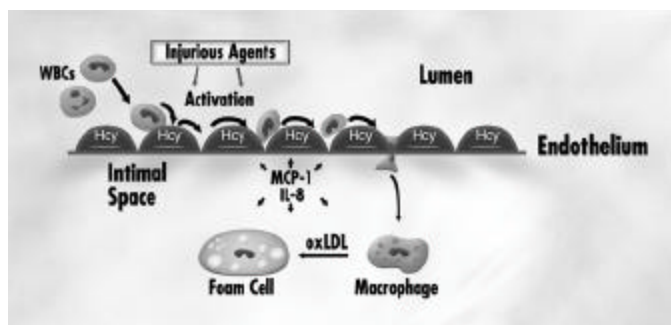


Figure 4. One proposed mechanism for homocysteine (Hcy) involvement in vascular disease. White blood cells (WBC) such as monocytes and neutrophils flowing through blood vessels normally have random contact with vascular endothelial cells (EC). When damage to ECs results from injurious agents, however, these WBCs begin to roll along, and adhere to, the endothelial surface. Hcy may speed the progression of vascular disease by stimulating production of monocyte and neutrophil chemoattractants—MCP-1 and IL-8—in the vascular endothelium. Secretion is targeted to the bottom side of the cell, thereby establishing a concentration gradient for chemotaxis. Once attached, monocytes migrate between ECs and become resident in the vascular intimal space. Here they are transformed into macrophages, engulf oxidized low-density lipoprotein (LDL), and become foam cells (the early observed lesion called a fatty streak). Foam cells are a source of reactive oxygen species which can play a role in other sequences of events that promote atherosclerosis.

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Additional Reading

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