Hyperhomocysteinemia and Thrombosis

An Overview

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• ***Context.*—Homocysteine, a sulfur-containing amino acid, absent in natural diets, is a metabolic intermediary in transmethylation and transsulfuration reactions. Such reactions are essential to normal cellular growth, differentiation, and function. Excess homocysteine is associated with vascular disease and related disorders.**

***Objective.*—To review homocysteine metabolism, the pathogenesis and classification of hyperhomocysteinemia, and the published literature investigating the association of homocysteine and methylenetetrahydrofolate reductase defects with arterial and venous thromboembolism and related disorders. The role of vitamin supplementation in patients with hyperhomocysteinemia is addressed.**

***Data Sources.*—Published medical and scientific literature. Articles addressing the objectives were selected and reviewed. Pertinent studies and conclusions were summarized, grouped, and contrasted.**

***Conclusions.*—The association of hyperhomocysteinemia and arterial and venous thrombosis is controversial. Severe hyperhomocysteinemia is associated with atherosclerosis. The effect of mild hyperhomocysteinemia is less certain. Coinheritance of methylenetetrahydrofolate reductase defects and factor V Leiden is likely to increase the risk of venous thromboembolism. The association of methylenetetrahydrofolate reductase defects combined with no additional thrombophilic risk factors with venous thrombosis is less clear. High doses of folic acid to lower homocysteine levels might not be necessary.**

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Homocysteine is a sulfur-containing amino acid absent in naturally occurring dietary sources. It is closely related to the essential amino acid methionine and to cysteine. Butz and Du Vigneaud[1] described the formation of homocystine from treating methionine with concentrated acid. Homocysteine is a metabolic intermediary in transmethylation and transsulfuration reactions. *S*-Adenosylmethionine (SAM), an intermediary in the methionine-homocysteine cycle, is an essential methyl donor in more than 100 known reactions including methylation of nucleic acids, proteins, phospholipids, myelin, polysaccharides, choline, and catecholamines. Impaired methylation is associated with abnormal cellular growth, differentiation, and function. The synthesis of glutathione, an important endogenous antioxidant, is dependent on the transsulfuration of homocysteine.

Aberrant homocysteine metabolism is associated with many disorders. In 1969, McCully[2] first described the association between homocystinemia and premature atherosclerotic vascular disease in homocystinuria. Presently, hundreds of publications discuss abnormal plasma homocysteine levels and various diseases. Hyperhomocysteinemia increases the likelihood of developing atherosclerosis. Hyperhomocysteinemia, alone or with other thrombophilic risk factors, may be associated with vascular occlusive pathology underlying varied clinical presentations.

Coronary vascular disease, stroke, recurrent pregnancy loss, and deep vein thrombosis are some of the presentations. Dementia, depression, retinal artery thrombosis, acquired hypercoagulable states after renal transplant, thrombosis in hemodialysis patients, Parkinson disease, thrombosis in diabetic patients, and acquired thrombophilia in systemic lupus erythematosus are among published disorders associated with hyperhomocysteinemia.

Collectively, the published studies suggest that elevated plasma homocysteine is injurious to blood vessels leading to vascular occlusive phenomena. Possible pathogenetic mechanisms of the vascular changes have been described. The causes of hyperhomocysteinemia range from aging and vitamin deficiency to genetic defects. Ultimately, the question of disease prevention and management of associated clinical presentations is debated. We review and summarize the published literature to present the current understanding of the relationship of homocysteine and thrombosis and the role of vitamin supplementation in thrombosis prevention.

HOMOCYSTEINE METABOLISM

Plasma levels of homocysteine are controlled by 2 distinct metabolic pathways: remethylation of homocysteine to methionine or transsulfuration of homocysteine to cysteine.[3] Homocysteine is formed intracellularly from the demethylation of dietary methionine, an essential amino acid, in the methionine cycle (Figure).[4] Homocysteine may acquire a methyl group from either *N*-5-methyltetrahydro-

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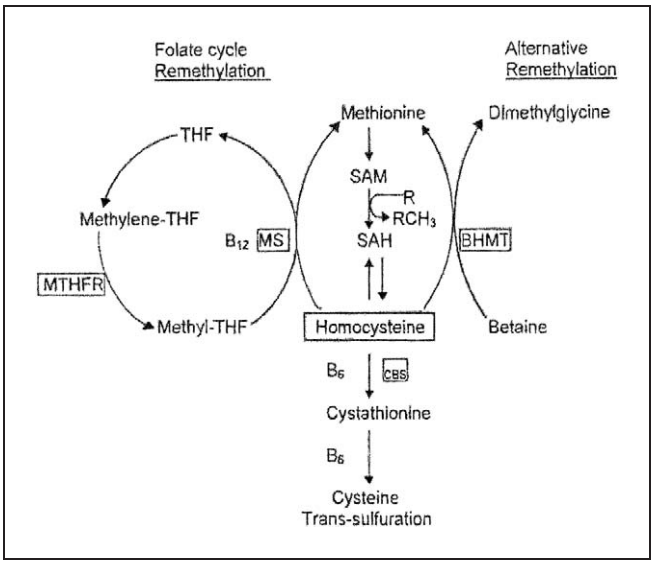

Schematic representation of homocysteine metabolism. THF indicates tetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; MS, methionine synthase; SAM, S*-adenosylmethionine; SAH,* S*-adenosylhomocysteine; BHMT, betaine homocysteine methyltransferase; CBS, cystathionine β-synthase; B_{12}, vitamin B_{12}; and B_6, vitamin B_6.*

folate (MTHF) an intermediary in the folate cycle or from betaine to re-form methionine (Figure). The folate cycle, essential for the MTHF reactions, occurs in all tissues and is vitamin B_{12} dependent.[3] Betaine is essentially confined to the liver and possibly the kidneys.[5–10]

Methylenetetrahydrofolate reductase (MTHFR) reduces 5,10-methylenetetrahydrofolate, in the folate cycle, to MTHF. The latter is converted to methionine by methyltransferase (methionine synthase) (Figure).[6] Methionine is preferentially activated by adenosine triphosphate to form SAM.[7] *S*-Adenosylmethionine is the universal methyl group donor. *S*-Adenosylhomocysteine is formed when SAM donates the methyl group; *S*-adenosylhomocysteine is hydrolyzed to regenerate homocysteine, propagating the methionine cycle.[3]

Betaine, in the alternative methionine remethylation pathway, helps the folate cycle in sustaining the methionine cycle and the production of SAM. Homocysteine is diverted to the transsulfuration pathway when methionine concentration exceeds the methionine cycle, folate cycle remethylation, capacity or when the synthesis of cysteine is required (Figure).[8,9] The initial step in transsulfuration is the union of homocysteine and serine forming cystathionine, catalyzed by cystathionine β-synthase (CBS). Pyridoxal 5′-phosphate (vitamin B_6) is an essential cofactor for CBS. Cystathionine is hydrolyzed by γ-cystathionase to form cysteine and α-ketobutyrate. Excess cysteine is oxidized to taurine or organic sulfates or is excreted in the urine. Therefore, transsulfuration not only is important for the synthesis of cysteine but serves to catabolize homocysteine in excess of the methionine cycle.[3] Transsulfuration regulates higher homocysteine concentrations, as in the postprandial state or after methionine loading. Remethylation, the main metabolic pathway of homocysteine, is responsible for the fasting plasma levels.[3]

Factors that can influence plasma homocysteine levels are genetic and acquired (Table 1).

Table 1. Factors That Influence Plasma Homocysteine Levels*

Acquired

1. Folate deficiency
   a. Dietary inadequacy
   b. Malabsorption
   c. Metabolic disorders, including alcohol and drugs
   d. Increased requirements and increased losses
2. Cobalamin deficiency
   a. Dietary inadequacy
   b. Gastrointestinal disorders
   c. Metabolic and transport disorders
3. Vitamin B_6 deficiency
   a. Inadequate supply
   b. Vitamin B_6 antagonists: natural antagonists and drug-B_6 interactions
4. Disease associated with hyperhomocysteinemia
   a. Kidney dysfunction
   b. Proliferative disorders: cancer, psoriasis
   c. Rheumatoid arthritis and systemic lupus
   d. Hypothyroidism
5. Drugs
   a. Hormones: sex hormones, insulin
   b. Antiepileptic drugs
   c. Nitrous oxide
   d. Lipid-lowering drugs
   e. Metformin
   f. Disulfide exchangers (D-penicillamine)
   g. Gastric proton pump inhibition
   h. Vitamin B_6 antagonists
   i. Methyl group acceptors (L-dopa, 6-mercaptopurine)
   j. Other drugs (sulfasalazine, megadoses of vitamin C)
6. Miscellaneous
   a. Increasing age
   b. Male sex
   c. Gastroplasty
   d. Down syndrome
   e. Increased muscle mass
   f. Carbon monoxide, cyanide
7. Lifestyle factors
   a. Exercise
   b. Smoking
   c. Alcohol consumption
   d. Coffee intake
   e. Vitamin intake
   f. Protein intake

Genetic

1. Cystathionine β-synthase deficiency
2. Inborn errors of folate metabolism
   a. Hereditary folate malabsorption
   b. Methylenetetrahydrofolate reductase deficiency (MTHFR)
   c. Glutamate formiminotransferase deficiency
3. Inborn errors of cobalamin absorption and transport
   a. Transcobalamin I (haptocorrin, R binder) deficiency
   b. Intrinsic factor deficiency
   c. Defective cobalamin transport by enterocytes (Imerslund-Gräsbeck syndrome)
4. Inborn errors of cobalamin metabolism
   a. Adenosylcobalamin deficiency
   b. Combined adenosylcobalamin and methylcobalamin deficiencies
   c. Methylcobalamin deficiency-methionine synthase reductase deficiency and methionine synthase deficiency
5. Polymorphism of folate and cobalamin metabolism
   a. Methylenetetrahydrofolate reductase
   b. Methionine synthase
   c. Methionine synthase reductase

* From Carmel and Jacobsen.[190]

Table 2. Disorders Associated With Hyperhomocysteinemia

1. Atherosclerosis (carotid artery intima-media thickening)
2. Coronary artery disease
3. Cerebral vascular disease
4. Peripheral arterial disease
5. Venous thromboembolic disease
6. Disordered hemostasis
   a. Platelet dysfunction: increased thromboxane A_2 synthesis
   b. Procoagulant activity: increased factor VIIIc, von Willebrand factor, thrombin-antithrombin complexes and prothrombin F1 and 2, and decreased factor VII
   c. Decreased natural anticoagulant activity
      1. Deficiency of antithrombin
      2. Deficiency of protein C

THE PATHOGENESIS OF HYPERHOMOCYSTEINEMIA

Homocysteine is metabolized intracellularly. A proportion, normally small, of homocysteine is released into the circulation. This release process and the transsulfuration pathway prevent the intracellular accumulation of this cytotoxic sulfur amino acid.[11,12]

Hyperhomocysteinemia occurs when the kidneys fail to excrete homocysteine or when a metabolic defect results in excess homocysteine entering the bloodstream. A genetic defect in one of the enzymes or a nutritional deficiency of cofactors (vitamins) in the remethylation or transsulfuration pathways can be associated with abnormal intracellular homocysteine levels and hyperhomocysteinemia.[3] Methionine synthase uses methylcobalamin (a vitamin B_{12} derivative) as a cofactor. Methylenetetrahydrofolate reductase uses flavin adenine dinucleotide (a riboflavin derivative) as a cofactor. Both CBS and cystathionase use pyridoxal phosphate (a vitamin B_6 derivative) as a cofactor.[13] Defects in any of these enzymes or cofactors are known to cause hyperhomocysteinemia. However, the severity of hyperhomocysteinemia appears to correlate with the specific abnormality. Genetic defects of MTHFR leads to impaired synthesis of MTHF, the first step in the synthesis of methionine.[3] Folate deficiency has a similar consequence. The hyperhomocystinemia resulting from impaired homocysteine remethylation because of deficiency in vitamin B_{12} or methionine synthase (methyltransferase) may not be as severe as observed in MTHFR defects, because transsulfuration will be somewhat more active in the catabolism of homocysteine.[3,14]

Abnormalities of the remethylation pathways do not alter the transsulfuration pathway. Abnormalities of the transsulfuration pathway, on the other hand, can affect the remethylation pathway.[3] In homozygous CBS defect, transsulfuration is severely impaired and homocysteine is diverted toward the remethylation pathway.[15] Methionine synthesis and consequently the intracellular concentration of SAM are increased. The folate remethylation pathway is inhibited when the intracellular concentration of SAM is sufficient for a feedback inhibition of MTHFR. Therefore, severe hyperhomocysteinemia associated with severe impairment of transsulfuration results in the inhibition of the folate remethylation pathway. When homocysteine level is low, as in fasting state, vitamin B_6 deficiency and heterozygous defect of CBS lead to a mildly impaired transsulfuration pathway, which together with the remethylation pathway prevent hyperhomocysteinemia. However, when the homocysteine burden is high, as in a significant dietary intake of methionine or the oral methionine load test, hyperhomocysteinemia results because homocysteine remethylation is inhibited through the feedback inhibition of MTHFR because of the increase in SAM. Further, in this situation homocysteine generation is accelerated through glycine methylation because glycine N-methyltransferase becomes highly active, as the result of loss of inhibitory action of MTHF secondary to MTHFR inhibition.[3]

Disorders possibly associated with hyperhomocysteinemia are listed in Table 2.

CLASSIFICATION OF HYPERHOMOCYSTEINEMIA

Hyperhomocysteinemia can be divided into 3 groups based on severity and pathogenetic mechanisms (Table 3).[3]

Severe hyperhomocysteinemia cases are due to homozygous defects in genes encoding for homocysteine metabolism. Homozygous defects in the gene encoding for CBS results in congenital homocystinuria.[16] Such patients usually present in childhood but occasionally as late as the seventh decade. One or more alerting signs might be present: dislocation of ocular lenses, lenticular myopia, Marfan-like appearance, thrombosis or thromboembolism, early-onset atherosclerosis, and mental retardation.

Vitamin B_6 supplementation can profoundly influence the clinical picture and the plasma levels of homocysteine and methionine in congenital homocystinuria. Therefore, patients should be off vitamin B_6 supplements for at least 1 to 2 weeks before sample collection. The expected fasting total plasma homocysteine values in CBS-deficient patients are usually more than 50 μmol/L (usually in the range of 100–500 μmol/L). Methionine levels are usually elevated, more than 40 μmol/L and may reach several hundreds of μmol/L. Rare mutations of MTHFR, methionine synthase, and methionine synthase reductase can cause homocystinuria.[15–19]

Homozygous defects in genes encoding for MTHFR or for any of the enzymes involved in vitamin B_{12} metabolism can lead to moderate to severe hyperhomocysteinemia. Severe MTHFR deficiency because of autosomal recessive in-

Table 3. Classification of Hyperhomocysteinemia

Type	Findings*
Severe–moderate hyperhomocysteinemia	High total homocysteine levels at all times; deficiencies in CBS, MTHFR, or in enzymes of B_{12} metabolism
Mild–moderate hyperhomocysteinemia	Moderately high total homocysteine levels under fasting conditions; reflects impaired homocysteine methylation (folate, B_{12}, or moderate enzyme defects, eg, thermolabile MTHFR)
Post-methionine load	Abnormal increase in total homocysteine after methionine load. Abnormal net increase reflects impaired homocysteine transsulfuration (heterozygous CBS deficiency, B_6 deficiency)

* CBS indicates cystathionine β-synthase; MTHFR, methylenetetrahydrofolate reductase.

Table 4. Polymorphic Mutations in 5,10-Methylenetetrahydrofolate Reductase			
Mutation	**Change in Amino Acid or Splice Site**	**Exon or Intron**	**Reference**
677C→T	Alanine/valine	Exon 4	20
1068T/C	Serine/serine	Exon 6	21
1178+31T/C	5′ splice site	Intron 6	22
1317T/C	Phenylalanine/phenylalanine	Exon 7	25
1298A→C	Glutamine/alanine	Exon 7	23–25

Table 5. Homocysteine Effect on Various Coagulation Factors		
Factor/Process*	**Effect**	**Evidence in Literature (Reference No.)**
Tissue factor expression	Increase	Suggestive (191)
Factor VII activity	Increase	Inconsistent (192–194)
Thrombin generation	Increase	Suggestive (194–196)
Factor V activation	Increase	Suggestive (197, 198)
Fibrinogen modification	Present	Suggestive (199, 200)
Thrombomodulin expression	Decrease	Inconsistent (197, 201, 202)
Inactivation of factor Va	Decrease	Inconsistent (203–205)
TFPI activity	Increase	Inconsistent (206, 207)
tPA binding	Decrease	Suggestive (208, 209)
Plasmin generation	Decrease	Suggestive (199, 209, 210)

* TFPI indicates tissue factor pathway inhibitor; tPA, tissue plasminogen activator.

heritance is rare and has been described in about 50 patients, ranging in age from birth to adult life. Inborn errors of vitamin B_{12} metabolism associated with hyperhomocysteinemia include adenosylcobalamin deficiency, combined adenosylcobalamin and methylcobalamin deficiencies, and methylcobalamin deficiency (methionine synthase reductase deficiency and methionine synthase deficiency). The number of patients described with these methionine deficiencies is relatively small.[3,15]

The more common causes of hyperhomocysteinemia are polymorphisms of folate and cobalamin metabolism and folate or cobalamin deficiencies. The resulting hyperhomocysteinemia is mild to moderate. Polymorphism refers to the prevalence of a mutation at a frequency of 1.0% or greater of alleles in a population. Polymorphism of MTHFR, methionine synthase, and methionine synthase reductase has been described.

Five common mutations resulting in sequence changes in MTHFR have been described (Table 4).[20–25] The 677C→T substitution (alanine to valine) has been studied extensively.[20,26–31] The 1298A→C (glutamine to alanine) has been studied less often.[24,29,32] The 1068T/C (serine/serine), 1178+31TC (5′ splice site), and the 1317T/C (phenylalanine/phenylalanine) are not likely to be clinically significant.[21–25] The frequency of homozygous MTHFR 677C→T in North American whites is 10% to 15%.[20,30,31] It is more common in Hispanic Americans with reported frequency of 25%.[33] African Americans have the least frequency, 0% to 1%.[33,34] This mutation was identified by Frosst et al,[20] who demonstrated the sensitivity of this variant to heat treatment at 46°C. Kang et al[26] and Engbersen et al[35] identified this thermolabile MTHFR in coronary artery disease patients by enzymatic assays of lymphocyte extracts. This mutation decreases specific activity of MTHFR at 37°C. Several studies demonstrated an association of the 677C→T mutation and hyperhomocysteinemia.[20,27,28,31,36,37] Guttormsen et al[36] identified 73% homozygosity in Norwegian individuals who were selected to have homocysteine levels greater than 40μM. The association between the 677C→T mutation and hyperhomocysteinemia is noted predominantly when the plasma folate level is low.[30] Folate supplementation to raise plasma folate levels above the median value can prevent hyperhomocysteinemia. The increase in folate levels might stabilize the mutant enzyme and allow it to function normally or provide exogenous MTHF for the remethylation pathway.[36,38,39] Methylenetetrahydrofolate reductase deficiency is associated with cardiovascular disease. This association is further magnified in the presence of other risk factors such as hypertension and hyperlipidemia.[40,41] Patients with factor V Leiden and MTHFR homozygous mutation have a significantly increased risk of thrombosis.[42,43]. Neural tube defects such as spina bifida, preeclampsia, recurrent pregnancy loss, and placental abruption have all been described in association with this mutation.[29,44–52] Folate supplementation during pregnancy prevents the recurrence of neural tube defects.[47,48]

Homozygous MTHFR 677C→T decreases the risk of colorectal cancer in folate-replete individuals by 50%. In folate-deficient individuals, no protection is afforded and perhaps the risk is enhanced.[53–55]

Methionine synthase 2756A→G mutation homozygosity is found to have frequency less than 5%. This polymorphism does not appear to be associated with hyperhomocysteinemia or an increased risk of neural tube defect or vascular disease.[56–59]

Methionine synthase reductase 66A→G mutation is extremely common. Wilson et al[60] reported a homozygosity frequency of 25% to 30% in the Canadian population. An increased risk of spina bifida was found in homozygous mutants, but no association with mild hyperhomocysteinemia was observed.

MEASURING HOMOCYSTEINE PLASMA LEVELS AND ASSESSING MTHFR STATUS

Pathologic homocysteine plasma levels requiring medical intervention are related to the normal plasma reference range, specimen type (fasting, random, or post-methionine load [PML] test), pretesting specimen handling, and test method. The latter 2 issues are not addressed other than to suggest following the manufacturer recommendations.

Several non–laboratory-related preanalytical variables affect homocysteine plasma levels (Table 1).

Healthy adults without any of the preanalytical variables that affect the plasma homocysteine level should be used in setting the reference range. The reference range varies in the literature and should be determined by individual laboratories.

Plasma homocysteine level is affected by the protein content in food intake. Therefore, a fasting specimen might be more informative, especially in setting the reference range. However, a different approach might be to order a fasting homocysteine level when a random specimen is abnormal. The evidence in the literature supports the finding that PML homocysteine testing identifies a subset of individuals with normal fasting homocysteine levels but abnormal PML tests. Such patients are likely to have a heterozygous genetic defect, MTHFR polymorphisms being the most frequent and probable cause.

Post-methionine load is impractical and not routinely offered. Post-methionine load is a global test for homocysteine metabolism. Therefore, PML would likely be abnormal in genetic abnormalities of homocysteine metabolism other than MTHFR polymorphisms. Individuals with MTHFR polymorphisms who are taking vitamin supplements might have homocysteine plasma levels within the reference range. Further investigation to determine if assessing the MTHFR status is a useful alternative to PML and to diagnose covert hyperhomocysteinemia is needed. Although there is lack of agreement in prospective and meta-analysis studies as to the association of hyperhomocysteinemia with arterial thrombosis and venous thromboembolism, retrospective case-control studies favor such association. Further, many publications suggest that homocysteine is injurious to the endothelium via a variety of mechanisms. Therefore, it seems prudent to include measuring plasma homocysteine levels and assessing MTHFR status in initial thrombophilia workup, until such time when solid evidence against this approach is introduced in the literature.

INJURIOUS MECHANISMS OF HYPERHOMOCYSTEINEMIA

Hyperhomocysteinemia is implicated in a wide spectrum of disorders: vascular damage, cognitive impairment, psychiatric and neurologic complications, congenital defects, pregnancy complications, and neoplastic disorders.[61–80] There are common underlying pathogenetic mechanisms associated with vascular injury leading to these clinical changes. The proposed pathogenetic mechanisms are oxidative damage of the endothelium through suppression of the vasodilator nitric oxide,[81–92] increasing the levels of asymmetric dimethylarginine, and impaired methylation,[89,93–98] vascular smooth muscle proliferation,[88,99–103] promotion of platelet activation and aggregation,[89,104–109] and disruption of the normal procoagulant-anticoagulant balance favoring thrombosis.[107,110–117]

Hyperhomocysteinemia promotes endothelial oxidative damage and dysfunction.[81–92,118,119] This might explain one of the benefits of antioxidant therapy.[83–85] Homocysteine inhibits endothelial nitric oxide synthase and subsequently the bioavailability of nitric oxide is markedly decreased resulting in impaired vasodilation.[89,98,106]

Nitric oxide detoxifies homocysteine by forming *S*-nitroso-homocysteine.[92] *S*-Nitroso-homocysteine is a vasodilator.[88,92,98,106] Auto-oxidation of excess homocysteine produces free radicals toxic to endothelial cells.[86,91,106] Normally, glutathione neutralizes free radicals. However, excess homocysteine decreases glutathione peroxidase activity.[89,90,118,119] An additional postulated mechanism of endothelial injury is through the diminished catabolism of asymmetric dimethylarginine. Asymmetric dimethylarginine is a strong inhibitor of nitric oxide synthase.[89,93–98] Hyperhomocysteinemia can directly impair DNA methylation resulting in altered gene expression, which may affect both the endothelial and smooth muscle cells of the vascular wall.[103,120] Several reports suggest that homocysteine induces proliferation of the vascular smooth muscle cells leading to luminal narrowing.[120,121] Excess homocysteine may be converted to the cyclic thioester homocysteine-thiolactone. Low-density lipoprotein may form adducts with homocysteine-thiolactone, which are phagocytized by macrophages and incorporated into foam cells in early atherosclerotic plaques.[88]

Platelets have normal life span and morphology in patients with hyperhomocysteinemia. However, homocysteine might activate platelets, increasing platelet aggregation and adhesion. Platelet thromboxane A_2 biosynthesis is significantly increased in homocystinuria. The enhanced production of thromboxane A_2 may be a major contributor to the risk of thrombosis.

Homocysteine rapidly auto-oxidizes in plasma. Free oxygen radicals are produced, which initiate lipid peroxidation either in endothelial plasma membrane or in lipoproteins. Oxidized low-density lipoprotein activates platelets and is atherogenic.

Several reports show that homocysteine promotes thrombosis by disturbing the procoagulant-anticoagulant balance. Homocysteine either increases or decreases several coagulation factors (Table 5).

HYPERHOMOCYSTEINEMIA AND THROMBOPHILIA

A large number of epidemiologic and experimental studies have investigated the association of hyperhomocysteinemia and thrombophilia. Epidemiologic studies addressing hyperhomocysteinemia and arterial or venous thrombosis included retrospective case-control, cross-sectional studies and prospective studies. Prospective vitamin therapy clinical trials to address whether hyperhomocysteinemia is a risk factor of atherothrombosis are ongoing. Case-control studies of genetic abnormalities of homocysteine metabolism and atherothrombosis and venous thrombosis have been done.

HYPERHOMOCYSTEINEMIA AND ARTERIAL DISEASE

McCully[2,122] observed premature atherosclerosis in homocysteinemia. Wilcken and Wilcken[123] provided evidence implicating homocysteine in coronary artery disease. Several subsequent studies reported an association between mild hyperhomocysteinemia and coronary artery disease, stroke, and peripheral arterial disease.[124–127] Other studies suggested that hyperhomocysteinemia was independent of established risk factors such as smoking, hyperlipidemia, hypertension, and diabetes for vascular occlusive disease.[120,121,128–130] Boushey et al[124] conducted a meta-analysis of 27 retrospective case-control studies addressing the association of hyperhomocysteinemia and vascular thrombotic disease. This analysis demonstrated that a 5-μmol/L incremental rise in total plasma homocysteine levels is associated with an increase in the relative risk for coronary artery disease, cerebrovascular disease, and pe-

Table 6. Hyperhomocysteinemia and Arterial Occlusive Disease; Studies Showing Correlation	
Study	**Findings***
Boushey et al[124]	5-μmol/L rise in total plasma HC increases relative risk of CAD, CVD, PVD
European Concerted Action Project[125]	HHC associated with increased risk of vascular disease multiplicative to other risk factors
Stampfer et al[136]	Relative risk of MI of 3.1 when HC levels were in the 95th percentile of control values
Malinow et al[137] and Voutilainen et al[127]	Increased plasma HC levels are associated with thickened carotid wall
Nygard et al[144]	Strong graded relationship between total HC and mortality
Kluijtmans et al[153] and Mudd et al[15]	677C→T MTHFR is a genetic risk for CAD

* HC indicates homocysteine; CAD, coronary artery disease; CVD, cerebrovascular disease; PVD, peripheral vascular disease; HHC, hyperhomocysteinemia; MI, myocardial infarction; and MTHFR, methylenetetrahydrofolate reductase.

ripheral vascular disease of 1.6, 1.5, and 6.8, respectively. The European Concerted Action Project, a multicenter study of 750 patients with vascular disease and 800 controls, confirmed that hyperhomocysteinemia is associated with an increased risk of vascular disease.[125] This risk was independent of, but multiplicative to, other risk factors such as smoking and hypertension and additive to hypercholesterolemia. Additional analysis of the same study indicate that red cell folate levels below the 10th percentile and of vitamin B_6 below the 20th percentile of control subjects were independent risk factors for vascular disorders.[131] Robinson et al[132] and Folsom et al[133] showed that low vitamin B_6 (pyridoxal phosphate) was an independent risk factor for coronary artery disease. Both Boers et al[134] and Malinow et al[135] showed that hyperhomocysteinemia was associated with peripheral arterial occlusive disease. Stampfer et al,[136] in a prospective study of plasma homocysteine and risk of myocardial infarction (MI) in US physicians that included 14 916 subjects, revealed a relative risk for MI of 3.1 when homocysteine levels were in the 95th percentile of control values compared with those below the 90th percentile. Malinow et al[137] showed an odds ratio for a thickened carotid intimal wall of 3.15 for patients in the top quintile of plasma homocysteine levels (>10.5μM) compared with those in the lowest quintile (<5.88μM). Voutilainen et al[127] reported an increased common carotid artery intimal-media wall thickness in men but not in women with plasma homocysteine levels more than 11.5μM. Konechy et al[138] revealed an independent correlation between plasma homocysteine levels and aortic atherosclerosis. Studies by Wu et al,[139] Hopkins et al,[140] Dalery et al,[141] and Verhoef et al[142] indicated that homocysteine levels are a risk factor for familial and nonfamilial coronary artery disease. Their work, however, suggested vitamins, especially folate and B_6, rather than homocysteine levels may confer the risk for coronary artery disease. Verhoef et al[143] in a study of plasma total homocysteine, B vitamins, and risk of coronary atherosclerosis found a graded correlation between occlusive coronary artery disease and both fasting and PML homocysteine levels. Nygard et al[144] evaluated plasma homocysteine-associated mortality in patients with coronary artery disease. They found a strong graded relationship between total homocysteine and mortality independent of variables. In a prospective study, Wald et al[145] found higher homocysteine levels in the group that died of ischemic heart disease than in controls.

Other prospective studies shed doubt on the relationship of hyperhomocysteinemia and coronary artery disease. Alfthan et al[146] found no statistical difference in total plasma homocysteine levels in 191 subjects who developed MI during the 9-year follow-up and the control subjects. Additional reports using data from the Physicians' Health Study show that homocysteinemia is associated with a statistically insignificant relative risk to develop coronary artery disease, angina pectoris with subsequent coronary artery bypass surgery, and stroke.[143] Evans et al[147] found no association of plasma homocysteine levels and MI. Folsom et al[148] found that total homocysteine levels correlated with the risk of coronary artery disease in women but not in men. Although in women only the level of homocysteine was inversely correlated with the folate levels, that was the case for both men and woman with vitamin B_6 levels. Molgaard et al[149] and Robinson et al[63] reported an inverse relationship of plasma homocysteine with folate and with vitamin B_{12}, vitamin B_6, and folate levels, respectively. Robinson et al[63] showed that low vitamin B_6 was an independent risk factor for coronary artery disease. Rimm et al[150] findings are in accord with Robinson and reported that vitamin B_6 and folate levels were inversely related to the risk of coronary artery disease among women. Selhub et al[151] reported similar findings.

The large number of reports investigating the association of hyperhomocysteinemia and the risk of arterial disease show conflicting results. Although hyperhomocysteinemia is likely a risk for arterial disease, that risk appears to be greater and more significant in patients with existing cardiovascular disease or low vitamin B levels. To that end, Donner et al[152] reported low prevalence of hyperhomocysteinemia in patients with low cardiovascular risk profile.

Similarly the correlation of genetic abnormalities of homocysteine metabolism and the risk of cardiovascular disease is uncertain. Kluijtmans et al[41,153] and Mudd et al[15] reported that 677C→T MTHFR was a genetic risk factor for cardiovascular disease. Brattström,[154] on the other hand, reported the 677C→T MTHFR mutation is not a causal risk factor for cardiovascular disease.

Table 6 lists studies showing correlation between hyperhomocysteinemia and arterial occlusive disease, and Table 7 lists studies casting doubt on such correlation.

HYPERHOMOCYSTEINEMIA AND VENOUS THROMBOSIS

Falcon et al,[155] in 1994, reported a high prevalence of hyperhomocysteinemia in patients with juvenile venous thrombosis. In 2 subsequent studies by den Heijer et al,[156,157] hyperhomocysteinemia greater than the 95th percentile of the control range was a risk factor for deep vein thrombosis. This group reported that vitamin supplementation with folate alone or with folate, B_{12}, and pyridoxine reduced homocysteine levels. den Heijer's work showed that several patients with abnormal PML total plasma homocysteine levels had normal fasting levels and vice versa. Therefore, the combination of the 2 tests would identify a

Table 7. Hyperhomocysteinemia and Arterial Occlusive Disease; Studies Showing No Correlation	
Study	**Findings***
Alfthan et al[146]	No statistical difference between individuals who developed MI and those who did not
Verhoef et al[143]	No statistically significant relative risk to develop CAD, angina, and stroke
Evans et al[147]	No association between plasma HC levels and MI
Folsom et al[148]	Total HC levels correlate with CAD in women but not men
Brattström[154]	677C→T MTHFR is not a causal risk for CAD

* MI indicates myocardial infarction; CAD, coronary artery disease; HC, homocysteine; and MTHFR, methylenetetrahydrofolate reductase.

Table 8. Hyperhomocysteinemia and Venous Thrombosis; Studies Showing Correlation	
Study	**Findings***
Falcon et al[155]	High prevalence of HHC in juvenile VT
den Heijer et al[156,157]	HHC > 95th percentile of control range is a risk factor for DVT
Simioni et al[158]	Significant high prevalence of HHC in patients with DVT of upper extremities
Eichinger et al[160]	• HHC in 25% of patients with a single episode of idiopathic VT • 2.7 risk of recurrent TE in the first 24 months after discontinuation of anticoagulation
Kottke-Marchant et al[126]	Plasma HC > 13 μM is a risk factor for arterial and venous thrombosis in patients with normal coagulation profiles
Fermo et al[162]	Moderate HHC in 13.1% of patients with VT and 19.2% of patients with AOD
den Heijer et al[161]	HHC associated with a calculated pooled odds ratio of 2.6 for VTE
Arruda et al,[165] Salomon et al,[166] and Margaglione et al[167]	Evidence in support of 677C→T MTHFR being a risk factor for VT (slightly greater risk for VT in homozygous vs heterozygous genotype)
Kluijtmans et al[173]	677C→T MTHFR may be a risk factor for thrombosis in CBS-deficient patients
Lalouschek et al[174]	677C→T MTHFR increased risk of TIA or minor strokes

* HHC indicates hyperhomocysteinemia; VT, venous thrombosis; DVT, deep venous thrombosis; TE, thromboembolism; HC, homocysteine; AOD, arterial occlusive disease; VTE, venous thromboembolism; MTHFR, methylenetetrahydrofolate reductase; CBS, cystathionine β-synthase; and TIA, transient ischemic attack.

larger group of individuals with abnormal homocysteine metabolism than either test alone. A case-control study by Simioni et al[158] identified a statistically significant high prevalence of hyperhomocysteinemia in patients with deep vein thrombosis. Martinelli et al[159] found no association of hyperhomocysteinemia and deep vein thrombosis of the upper extremities. Eichinger et al[160] found that hyperhomocysteinemia was present in 25% of 264 individuals with a single episode of idiopathic venous thromboembolism. This group identified that the risk of recurrent thromboembolism was 2.7 in the first 24 months after discontinuation of anticoagulation. In a prospective study by Kottke-Marchant et al,[126] high plasma homocysteine levels more than 13μM were found to be a risk factor for arterial and venous thrombosis in patients with normal coagulation profiles. An elevated homocysteine level yielded a 7.8 odds ratio for thrombosis. Women had a higher odds ratio than men.[126] In a quantitative review of hyperhomocysteinemia and venous thrombosis, den Heijer et al[161] calculated a pooled odds ratio for venous thrombosis of 2.6.

Fermo et al[162] detected moderate hyperhomocysteinemia in 13.1% of patients with venous and 19.2% of patients with arterial occlusive disease. Other heritable thrombophilic factors were present in same group of patients with venous thrombosis. Fermo et al calculated the relative risk of venous thrombosis in patients with combined hyperhomocysteinemia and other thrombophilic factors was 1.7 times greater than for patients with hyperhomocysteinemia alone. The age of occurrence of the first thrombotic episode was earlier in the subset of patients with combined risk factors. Ridker et al[163] reported a 10-fold increase in thrombotic risk among patients with both hyperhomocysteinemia and factor V Leiden. This group found that hyperhomocysteinemia conferred a relative risk of 3.4 in patients with idiopathic venous thrombosis.

Legnani et al[164] found no association between elevated fasting or PML homocysteine levels and thrombosis in a group of patients with protein C, protein S, or antithrombin deficiency or factor V Leiden. 677C→T MTHFR did not confer additional thrombotic risk to the heritable thrombophilic coagulation effects. Whether 677C→T MTHFR is a risk factor for venous thrombosis is debatable. The published studies show conflicting results. Arruda et al,[165] Salomon et al,[166] and Margaglione et al[167] show evidence in support of 677C→T MTHFR being a risk factor for venous thrombosis. De Stefano et al[168] reviewed 9 case-control studies involving 2225 patients with venous thrombosis and 2994 healthy controls. There were no significant differences in the cumulative prevalence between homozygous MTHFR genotype in cases with venous thrombosis versus normal controls. Only 2 studies showed a slightly greater risk for venous thrombosis in the homozygous genotype compared with heterozygous.[165–167] Nevertheless, Trillot et al[169] and others showed that 677C→T MTHFR does not modify the risk of venous thrombosis. Further, although Cattaneo et al[170,171] indicated that the coexistence of 677C→T MTHFR and factor V Leiden increased the risk of venous thrombosis, Trillot et al[169] and Kluijtmans et al[172] suggested that this mutation does not modify the risk for venous thrombosis in patients with heterozygous factor V Leiden. Kluijtmans et al[173] suggested the 677C→T MTHFR may be a risk factor for thrombosis in CBS-deficient patients. Lalouschek et al[174] reported an increased risk of transient ischemic attacks or minor strokes in patients with 677C→T MTHFR.

Table 8 lists studies supporting a correlation between hyperhomocysteinemia and venous thrombosis, and Table 9 lists studies with different conclusions. Table 10 summarizes studies addressing the effect of hyperhomocysteinemia combined with other thrombophilic risk factors.

Table 9. Hyperhomocysteinemia and Venous Thrombosis; Studies Showing No Correlation	
Study	**Findings***
Martinelli et al[159]	No association of HHC and DVT of upper extremities
Trillot et al[169] and Kluijtmans et al[172]	677C→T MTHFR does not modify risk of VT
De Stefano et al[168]	• 9 case-control studies involving 2225 patients with VT and 2994 healthy controls • No significant differences in cumulative prevalence between homozygous MTHFR in cases with VT vs normal controls

* HHC indicates hyperhomocysteinemia; DVT, deep venous thrombosis; MTHFR, methylenetetrahydrofolate reductase; and VT, venous thrombosis.

Table 10. Effect of Hyperhomocysteinemia Combined With Other Thrombophilic Risk Factors	
Study	**Findings***
Fermo et al[162]	The relative risk of VT in patients with HHC combined with other thrombophilic factors was 1.6 times greater than for patients with HHC alone and patients developed first thrombotic episode at a younger age
Ridker et al[163]	10-fold increase in thrombotic risk among patients with HHC and FVL
Legnani et al[164]	• No association between HHC and thrombosis in patients with protein C, protein S, AT def, or FVL • 677C→T MTHFR did not confer additional thrombotic risk factor to the heritable thrombophilic coagulation defects
Cattaneo et al[170,171]	Coexistence of 677C→T MTHFR and FVL increased risk of VT

* VT indicates venous thrombosis; HHC, hyperhomocysteinemia; FVL, factor V Leiden; AT def, antithrombin deficiency; and MTHFR, methylenetetrahydrofolate reductase.

Table 11. Norwegian Vitamin Trial; Event Rates (Per 1000 Person-Years)				
	Folic Acid + Vitamin B_6	**Folic Acid**	**Vitamin B_6**	**Placebo**
Primary end point	81.6	66.9	70.1	67.2
MI*	73.0	57.5	64.0	59.2
Death from any cause	37.5	28.7	33.4	31.7
Cancer	12.0	11.9	8.0	9.0

* MI indicates myocardial infarction.

TREATMENT OF HYPERHOMOCYSTEINEMIA

The conventional treatment of hyperhomocysteinemia has been folate supplementation usually with vitamin B_6 and perhaps vitamin B_{12}. Although this approach is successful in lowering total plasma homocysteine levels, its effect on clinical vascular pathology remained untested until recently.

The Norwegian Vitamin Trial, a randomized trial of homocysteine lowering with B vitamins for secondary prevention of cardiovascular disease after acute MI, has been completed. This is the largest trial testing the benefit of folate supplementation in reducing the risk of recurrent MI and reported its findings September 2005 at the European Society of Cardiology 2005 Congress.[175]

Although a 28% reduction of plasma homocysteine levels was achieved, there was no associated risk reduction for MI or stroke. There was not a significant effect on the risk for cardiovascular disease in patients taking either folic acid alone or vitamin B_6 alone. Interestingly a 21% increased risk of MI was found in patients taking folic acid and vitamin B_6 in combination. An increase in cancer was seen in patients taking either folic acid alone or folic acid and vitamin B_6 but not in those taking vitamin B_6 alone (Tables 11 and 12).

The Norwegian Vitamin Trial study suggests that homocysteine is an innocent bystander in patients with cardiovascular disease. It is important to point out that hyperhomocysteinemia was not an inclusion criterion in the Norwegian Vitamin Trial study. Many questions and possible hypotheses remain unanswered and untested.

The results of Vitamins to Prevent Stroke and Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine, 2 ongoing trials in large populations, should add more insight into the impact of folic acid supplementation in patients with cerebrovascular and ischemic heart disease. Several smaller studies did shed doubt on the usefulness of folic acid supplementation in patients with coronary artery disease.

In a randomized study of 593 patients with stable coronary artery disease, Liem et al[176] found that, within the follow-up time of 24 months, folic acid did not seem to reduce clinical end points in patients with stable coronary artery disease while on statin treatment. The authors concluded that homocysteine might be a modifiable marker of disease and that folic acid supplementation should be treated with reservations. In an outcome trial by Baker et al,[177] 1882 patients with evidence of coronary disease were randomized to folic acid or placebo in addition to the usual drugs for 2 years. The only predictors of outcome were plasma homocysteine and age. Although homocysteine was reduced from 11.2 ± 6.9 to 9.7 ± 5.3 μmol/L, there was no difference in composite outcome. There was a 2-fold difference in nonfatal MI (23 vs 12, $P = .05$) but no difference in deaths or revascularization. The authors conclude that routine use of folic acid supplementation in patients with ischemic heart disease and slight elevation of plasma homocysteine is not warranted. Lange et al[178] tested the effect of a combination of folic acid, vitamin B_6, and vitamin B_{12} on the risk of angiographic restenosis after coronary stent placement in a double-blind multicenter trial. A total of 636 patients were enrolled. The authors found at follow-up time a significantly smaller minimal luminal diameter, greater late luminal loss, and higher restenosis rate in the folate group compared with the placebo group. Repeated target-vessel revascularization was higher in the folate group. In the Vitamin Intervention for Stroke Pre-

Table 12. Norwegian Vitamin Trial; Rate Ratios*

	Folic Acid Vs Control			Vitamin B_6 Vs Control			Folic Acid + Vitamin B_6 Vs Control		
	RR	95% CI	*P*	RR	95% CI	*P*	RR	95% CI	*P*
MI and stroke	1.1	0.9–1.3	.30	1.1	1.0–1.3	.09	1.2	1.0–1.4	.03
MI	1.1	0.9–1.2	.50	1.2	1.0–1.4	.04	1.2	1.0–1.4	.03
Death	1.0	0.8–1.3	.80	1.2	1.0–1.5	.11	1.2	1.0–1.5	.10
Cancer	1.4	1.0–2.0	.08	1.0	0.7–1.4	.30	1.3	0.8–1.9	.30

* MI indicates myocardial infarction; RR, relative risk; and CI, confidence interval. Adapted from Bonaa et al.[211]

Table 13. Homocysteinemia and Thrombosis

1. Hyperhomocysteinemia is a cause of atherosclerosis and venous thrombosis.
2. Hyperhomocysteinemia is associated with either atherosclerosis or venous thrombosis but not both.
3. Hyperhomocysteinemia is not a cause but a marker of vascular disease, an innocent bystander.
4. Hyperhomocysteinemia is a risk factor for vascular disease only in very high concentrations.
5. Hyperhomocysteinemia is associated with vascular disease in patients with coexistent risk factors.
6. Hyperhomocysteinemia is a surrogate for low vitamin B levels, which is the true risk for vascular disease.

vention randomized controlled trials, Toole et al[179] tested the effect of lowering homocysteine in patients with ischemic stroke to prevent stroke, MI, and death. A total of 3600 adults with nondisabling cerebral infarction were enrolled. The authors concluded that a moderate reduction of total homocysteine had no effect on vascular outcomes during the 2 years of follow-up. Nevertheless, because there was a consistent association of total homocysteine with vascular risk, the authors suggest that further investigations are necessary. In October 2005, Lewis et al[180] published the largest meta-analysis of the association of MTHFR 677C→T polymorphism and coronary heart disease. The authors found no strong evidence to support an association of MTHFR 677C→T and coronary artery disease in Europe, North America, or Australia. Geographic variations exist. This study cast doubt on the role of supplemental folic acid in preventing cardiovascular disease, especially in high-income countries with folate-fortified food. It is important to note that some studies do show a beneficial effect of folic acid supplementations. Williams et al,[181] in a randomized placebo-controlled, double-blind study of 41 subjects, showed that a 3-week folic acid supplementation, but not placebo, resulted in a reduction of brachial artery pulse pressure by 4.7± 1.6 mm Hg (P = .05) without changing mean arterial pressure. Systemic arterial compliance increased by 0.15 ± 0.03 mL/mm Hg (P = .05). These results were independent of homocysteine or folate concentration and MTHFR genotype.

Assanelli et al[182] in a randomized trial in 30 young subjects with recent acute MI and high plasma homocysteine levels found that a marked reduction in plasma homocysteine concentrations is associated with a significant improvement of endothelial function independent of plasma antioxidant capacity.

Finally, the studies by Stott et al[183] and Nurk et al[184] published December 2005 are noteworthy. Stott et al studied 185 patients, 65 years or older, with ischemic vascular disease in a randomized, placebo-controlled, double-blind study with 3 active treatments: folic acid (2.5 mg) plus vitamin B_{12} (500 mg), vitamin B_6 (25 mg), and riboflavin (25 mg). Changes in plasma homocysteine, fibrinogen, and von Willebrand factor were measured at 3, 6, and 12 months and changes in cognitive functions at 6 and 12 months. The authors found that, although homocysteine levels decreased in the group receiving oral folic acid plus vitamin B_{12} supplementation, there was no statistically significant beneficial effects on cognition. Nurk et al[184] scrutinized the 2189 subjects in the Hordaland homocysteine study population measuring total homocysteine and folate levels and assessing memory performance using the Kendrick Object Learning Test at baseline and 6 years later. The authors conclude that increased plasma total homocysteine is an independent risk factor for memory deficit both cross-sectionally and prospectively. A favorable change in folate and or total homocysteine during time is associated with better cognitive performance.

Silaste et al[185] reported that a diet high in fresh berries, citrus fruit, and vegetables effectively increases serum and red blood cell folate and decreases plasma homocysteine. Several studies show that betaine and choline supplementation lower plasma homocysteine in healthy men and women.[186–188] *N*-Acetylcysteine therapy is another possible option.[189] Alternative methods to reduce plasma homocysteine might be worth pursuing.

In conclusion, the answer to the question—Is hyperhomocysteinemia a risk factor for vascular occlusive disease?—is a qualified affirmation. We suggest that there are several possible hypotheses relating hyperhomocysteinemia and thrombosis (Table 13).

Additional studies are needed to determine which of the 6 hypotheses is true. Hyperhomocysteinemia is related to atherosclerosis and disorders resulting from arterial vascular disease in a graded manner. This association is modulated by preexisting vascular disease, if any, vitamin levels, and other risk factors for cardiovascular disease.

The association of hyperhomocysteinemia and venous thrombosis is controversial. The interplay of aberrant homocysteine metabolism, vitamin levels, and other inherited coagulation defects are likely important factors contributing to the risk of thrombosis.

Should patients at risk for atherothrombosis or venous thrombosis receive folate supplementation? Perhaps, the most reasonable approach, given the current state of knowledge, is to treat hyperhomocysteinemia patients who have additional risk factors for atherothrombosis or venous thrombosis, including those with MTHFR homozygous 677C→T. Dietary treatment should be first attempted followed by either folate or folate alternatives (be-

taine, choline, *N*-acetylcysteine) supplementation. Folate alternative therapy should be considered in patients with higher risk for breast or prostate cancer.

To this end, it is reasonable to assume that the final verdict on folate supplementation has not been reached yet. More studies are needed to investigate various hypotheses and clinical situations. Meanwhile, a conservative approach to normalize plasma homocysteine levels might be best accomplished by a healthy diet of fresh fruit and vegetables and moderate exercise.

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