HYPERHOMOCYSTEINEMIA AND THROMBOSIS:

AN OVERVIEW
Abstract:

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 MTHFR 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. Data extraction and Synthesis: Articles addressing the objectives were selected and reviewed. Pertinent studies and conclusions summarized, grouped and contrasted. The prepared manuscript was reviewed by both authors. Conclusion: 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 MTHFR and Factor V Leiden is likely to increase the risk of venous thromboembolism. The association of MTHFR 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.
Introduction

Homocysteine is a sulphur 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 described the formation of homocystine from treating methionine with concentrated acid \(^1\). Homocysteine is a metabolic intermediary in transmethylation and transsulfuration reactions. S-Adenosylmethionine, an intermediary in the methionine-homocysteine cycle, is an essential methyl donor in over 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 Gultathione, an important endogenous antioxidant, is dependent on the transsulfuration of homocysteine.

Aberrant homocysteine metabolism is associated with many disorders. In 1969, McCully first described the association between homocystinemia and premature atherosclerotic vascular disease in homocystinuria \(^2\). 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's disease, thrombosis in diabetic patients, 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 two 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 (Fig. 1) \(^{(4)}\). Homocysteine may acquire a methyl group from either N-5-methyl-tetrahydrofolate (MTHF) an intermediary in the folate cycle, or from betaine to reform methionine (Fig. 1). The folate cycle, essential for the MTHF reactions, occurs in all tissues and is Vitamin B12 dependent \(^{(3)}\). Betaine is essentially confined to the liver and possibly the kidneys \(^{(5-10)}\).

75 Methylene tetrahydrofolate reductase (MTHFR) reduces 5,10 methylene – tetrahydrofolate, in the folate cycle, to 5-methyltetrahydrofolate. The latter is converted to methionine by methyltransferase (methionine synthase) (Fig. 1) \(^{(6)}\). Methionine is preferentially activated by ATP to form S-adenosylmethionine (SAM) \(^{(7)}\). SAM is the universal methyl group donor. S-adenosylhomocysteine (SAH) is formed when SAM donates the methyl group; SAH 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 \(^{(8-9)}\) (Fig. 1). The initial step in transsulfuration is the union of homocysteine and serine forming cystathionine, catalyzed by cystathionine B-synthase (CBS). Pyridoxal 5' – phosphate (Vitamin B\(_{6}\)) is an essential cofactor for CBS. Cystathionine is hydrolyzed by \(\gamma\)-cystathionase to form cysteine and \(\alpha\)-ketobutyrate. Excess cysteine is oxidized to taurine or organic sulfates or is excreted in
the urine. Therefore, not only is transsulfuration important for the synthesis of cysteine, but is
serves to catabolize homocysteine in excess of the methionine cycle (3). Transsulfuration
regulates higher homocysteine concentrations, as in the post prandial 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. These are
listed in Table 1.
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 Methyl cobalamin (a Vitamin B\(_{12}\) derivative) as a cofactor. MTHFR uses FAD (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 N-5-methyltetrahydrofolate, the first step in the synthesis of methionine \(^{(3)}\). Folate deficiency has a similar consequence. The hyperhomocysteinemia resulting from impaired homocysteine remethylation because of deficiency 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. 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 B₆ deficiency and heterozygous defect of cystathionine B-synthase lead to a mildly impaired transsulfuration pathway, which together with the remethylation pathway prevent hyperhomocysteinemia. However, when homocysteine burden is high, as in a significant dietary intake of methionine or the oral methionine lead test, hyperhomocysteinemia results because homocysteine remethylation is inhibited through the feedback inhibition of MTHFR due to the increase in SAM. Further, in this situation homocysteine generation is accelerated through glycine methylation because glycine N-methyltransferase (GNMT) becomes highly active, as the result of loss of inhibitory action of N-5-methyltetrahydrofolate secondary to MTHFR inhibition (3).

Disorders possibly associated with hyperhomocysteinemia are listed in Table 2.
Hyperhomocysteinemia can be divided into three groups based on severity and pathogenetic mechanisms (3) Table 3.

Severe hyperhomocysteinemia cases are due to homozygous defects in genes encoding for homocysteine metabolism. Homozygous defects in the gene encoding for Cystathionine beta synthase (CBS) results in congenital homocystinuria (16). Such patients present usually 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 B6 supplementation can profoundly influence the clinical picture and the plasma levels of homocysteine and methionine in congenital homocystinuria. Therefore patients should be off vitamin B6 supplements for at least 1 to 2 weeks before sample collection. The expected fasting total plasma homocysteine values in CBS deficient patients are usually >50 µmol/L (usually in the range of 100-500 µmol/L). Methionine levels are usually elevated, >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 B12 metabolism can lead to moderate to severe hyperhomocysteinemia. Severe MTHFR deficiency due to autosomal recessive inheritance is rare and has been described in about 50 patients, ranging in age from birth to adult life. Inborn errors of Vitamin B12 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-moderate. Polymorphism refers to the prevalence of a mutation at a frequency of \(\geq 1.0\%\) of alleles in a population. Polymorphism of methylenetetrahydrofolate reductase (MTHFR), methionine synthase and methionine synthase reductase, have 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 1317 T/C (phenylalanine/phenylalanine) are not likely to be clinically significant \(^{21-25}\). The frequency of homozygous MTHFR 677C→T in North American whites is 10-15% \(^{20, 30-31}\). It is more common in Hispanic Americans with reported frequency of 25% \(^{33}\). African Americans have the least frequency 0-1% \(^{33-34}\). This mutation was identified by Frosst et al, who demonstrated the sensitivity of this variant to heat treatment at 46°C \(^{20}\). Kang et al, and Engbersen et al, identified this thermolabile MTHFR in coronary artery disease patients by enzymatic assays of lymphocyte extracts \(^{26, 35}\). 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, identified 73% homozygosity in Norwegian individuals who were selected to have homocysteine levels greater than 40µM \(^{36}\). 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 5-methyltetrahydrofolate for the remethylation pathway \(^{(36, 38-39)}\). MTHFR 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, pre-eclampsia, 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, reported a homozygosity frequency of 25-30% in the Canadian population \(^{(60)}\). 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 is related to the normal plasma reference range, specimen type (fasting, random or post-methionine load test), pretesting specimen handling and test method. The latter two issues shall not be addressed other than to suggest following the manufacturer recommendations.

Several non-laboratory related pre-analytical variables affect homocysteine plasma levels.

Table 1.

Healthy adults without any of the pre-analytical 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 that post-methionine load (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.

PML is impractical and not routinely offered. PML 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. While 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 neurological 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 dimethylarginine (ADMA), 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 (NO) synthase and subsequently the bioavailability of NO is markedly decreased resulting in impaired vasodilation (106,89, 98).

NO detoxifies homocysteine by forming S-nitroso-homocysteine (SN) (92). SN is a vasodilator (88, 92, 98, 106). Autooxidation 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 (ADMA). ADMA is a strong inhibitor of NO 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 \cite{103,120}. Several reports suggest that homocysteine induces proliferation of the vascular smooth muscle cells leading to luminal narrowing \cite{120-121}. Excess homocysteine may be converted to the cyclic thioester homocysteine-thiolactone, (HSL). LDL may form adducts with HSL, which are phagocytized by macrophages and incorporated into foam cells in early atherosclerotic plaques \cite{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 lipoproteins. Oxidized LDL activate platelets and are atherogenic.

Several reports show that homocysteine promotes thrombosis by disturbing the procoagulant/anticoagulant balance. Homocysteine either increases or decreases several coagulation factors. Table 5.
A large number of epidemiological and experimental studies have investigated the association of hyperhomocysteinemia and thrombophilia. Epidemiological studies addressing hyperhomocysteinemia and arterial or venous thrombosis included retrospective case—control and 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.
McCully observed premature atherosclerosis in homocysteinemia. Wilcken and Wilcken 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. Other studies suggested that hyperhomocysteinemia was independent of established risk factors such as smoking, hyperlipidemia, hypertension and diabetes for vascular occlusive disease. Boushey et al conducted a meta-analysis of 27 retrospective case-control studies addressing the association of Hyperhomocysteinemia and vascular thrombotic disease. This analysis demonstrated that a 5µmol/L incremental rise in total plasma homocysteine levels is associated with an increase in the relative risk for coronary artery disease, cerebrovascular disease and peripheral vascular disease of 1.6, 1.5 and 6.8 respectively. The European Concerted Action Project, a multi-center study of 750 patients with vascular disease and 800 controls confirmed that hyperhomocysteinemia is associated with an increased risk of vascular disease. This risk was independent of, but multiplicative to other risk factors such as smoking, hypertension and additive to hypercholesterolemia. Additional analysis of the same study indicate that red cell folate levels below the 10th percentile and of Vitamin B6 below the 20th percentile of control subjects were independent risk factors for vascular disorders. Robinson et al, and Folsom et al, showed that low vitamin B6 (pyridoxal-phosphate) was an independent risk for coronary artery disease. Both Boers et al, and Malinow et al, showed that hyperhomocysteinemia were associated with peripheral arterial occlusive disease. Stampfer et al, in a prospective study of plasma homocysteine and risk of myocardial infarction in US physicians, that included 14, 916 subjects, revealed a relative risk for myocardial infarction of 3.1 when homocysteine levels were in the 95th percentile of control values compared to those below the 90th percentile. Malinow et al, showed, an odds ratio
for a thickened carotid intimal wall of 3.15 for patients in the top quintile of plasma homocysteine levels (>10.5 µM) compared to those in the lowest quintile (<5.88 µM)\(^{(137)}\).

Voutilainen et al, reported an increased common carotid artery intimal-media wall thickness in men but not women with plasma homocysteine levels >11.5 µM\(^{(123)}\). Konechy et al, revealed an independent correlation between plasma homocysteine levels and aortic atherosclerosis\(^{(138)}\). Studies by Wu, Hopkins, Dalery and Verhoef indicate that homocysteine levels are a risk factor for familial and non-familial coronary artery disease\(^{(139-142)}\). Their work, however, suggests vitamins, especially folate and B\(_6\), rather than homocysteine levels may confer the risk for coronary artery disease. Verhoef et al, 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 post methionine load homocysteine levels\(^{(143)}\).

Nygard et al, evaluated plasma homocysteine associated mortality in patients with coronary artery disease\(^{(144)}\). They found a strong graded relationship between total homocysteine and mortality independent of variables. In a prospective study Wald et al, found higher homocysteine levels in the group that died of ischemic heart disease than in controls\(^{(145)}\).

Other prospective studies shed doubt on the relationship of hyperhomocysteinemia and coronary artery disease. Alfthan et al, found no statistical difference in total plasma homocysteine levels in 191 subjects who developed myocardial infarction during the 9-year follow up and the control subjects\(^{(146)}\). Additional reports utilizing 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, found no association of plasma homocysteine levels and myocardial infarction\(^{(147)}\). Folsom et al, found that total homocysteine levels correlated with the risk of coronary artery disease in women but not in men. While 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 B₆ levels. Molgaard et al, and Robinson et al, reported an inverse relationship of plasma homocysteine with folate and with vitamin B₁₂, Vitamin B₆ and folate levels respectively. Robinson showed that low vitamin B₆ was an independent risk factor for coronary artery disease. Rimm et al, findings are in accord with Robinson and reported that vitamin B₆ and folate levels were inversely related to the risk of coronary artery disease among women. Selhub et al, report similar findings.

The large number of reports investigating the association of hyperhomocysteinemia and the risk of arterial disease show conflicting results. While 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, 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, and Mudd et al, reported that 677C→T MTHFR was a genetic risk factor for cardiovascular disease. Brattström et al, 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, while Table 7 lists studies casting doubt on such correlation.
Falcon et al, in 1994, reported a high prevalence of hyperhomocysteinemia in patients with juvenile venous thrombosis (155). In two subsequent studies by den Heijer et al, hyperhomocysteinemia greater than the 95th percentile of the control range was a risk factor for deep-vein thrombosis (156-157). This group reported that vitamin supplementation with folate alone, or with folate, B12, and pyridoxine reduced homocysteine levels. den Heijer work showed that several patients with abnormal post-methionine loading total plasma homocysteine levels had normal fasting levels and vice versa. Therefore, the combination of the two tests would identify a larger group of individuals with abnormal homocysteine metabolism than either test alone. A case control study by Simioni et al, identified a statistically significant high prevalence of hyperhomocysteinemia in patients with deep-vein thrombosis (158). Martinelli et al, found no association of hyperhomocysteinemia and deep-vein thrombosis of the upper extremities (159). Eichinger et al, 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 (160). In a prospective study by Kottke-Marchant et al, high plasma homocysteine levels >13µM was found to be a risk factor for arterial and venous thrombosis in patients with normal coagulation profiles (126). 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, calculated a pooled odds ratio for venous thrombosis of 2.6 (161).
Fermo et al, detected moderate hyperhomocysteinemia in 13.1% of patients with venous and 19.2% of patients with arterial occlusive disease \(^{(162)}\). 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, reported a tenfold increase in thrombotic risk among patients with both hyperhomocysteinemia and Factor V Leiden \(^{(163)}\). This group found that hyperhomocysteinemia conferred a relative risk of 3.4 in patients with idiopathic venous thrombosis.

Legnani et al, found no association between elevated fasting or post-methionine load homocysteine levels and thrombosis in a group of patients with protein C, protein S or antithrombin deficiency or Factor V Leiden \(^{(164)}\). 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, Salamon et al, and Margaglione et al, show evidence in support of 677C→T MTHFR being a risk factor for venous thrombosis \(^{(165-167)}\). De Stefano et al, reviewed nine case-control studies involving 2,225 patients with venous thrombosis and 2,994 healthy controls. There were no significant differences in the cumulative prevalence between homozygous MTHFR genotype in cases with venous thrombosis versus normal controls \(^{(168)}\). Only two studies showed a slightly greater risk for venous thrombosis in the homozygous genotype compared to heterozygous \(^{(165-167)}\). Nevertheless, Trillot et al, and others show that 677C→T MTHFR does not modify the risk of venous thrombosis \(^{(169)}\). Further, while Cattaneo et al, indicate that the coexistence of 677C→T MTHFR and Factor V Leiden increased the risk
of venous thrombosis, Trillot et al., and Kluijtmans et al., suggest that this mutation does not modify the risk for venous thrombosis in patients with heterozygous Factor V Leiden (169-172). Kluijtmans et al., suggest the $677C \rightarrow T$ MTHFR maybe a risk factor for thrombosis in CBS-deficient patients (173). Lalouschek et al., reported an increased risk of transient ischemic attacks or minor strokes in patients with $677C \rightarrow T$ MTHFR (174).

Table 8 lists studies supporting a correlation between hyperhomocysteinemia and venous thrombosis, while Table 9 lists studies with different conclusions. Table 10 summarizes studies addressing the effect of hyperhomocysteinemia combined with other thrombophilic risk factors.
The conventional treatment of hyperhomocysteinemia has been folate supplementation usually with Vitamin B₆ and perhaps vitamin B₁₂. While this approach is successful in lowering total plasma homocysteine levels, its effect on clinical vascular pathology remained untested until recently.

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

While 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 B₆ alone. Interestingly a 21% increased risk of MI was found in patients taking folic acid and vitamin B₆ in combination. An increase in cancer was seen in patients taking either folic acid alone or folic acid and vitamin B₆, but not in those taking vitamin B₆ alone. Tables 11 and 12.

The NORVIT 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 NORVIT study. Many questions and possible hypotheses remain unanswered and untested.
The results of VITATOPS (Vitamins to Prevent Stroke) and SEARCH (Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine) two 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, found that, within the follow-up time of 24 months, folic acid did not seem to reduce clinical endpoints in patients with stable coronary artery disease, while on statin treatment \(^{(176)}\). The authors conclude 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, 1,882 patients with evidence of coronary disease were randomized to folic acid or placebo in addition to the usual drugs for two 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 \(\mu\text{mol/L}\), there was no difference in composite outcome. There was a twofold difference in non-fatal myocardial infarction \(23 \text{ vs } 12, P=.05\) 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 \(^{(177)}\). Lange et al, 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 multi-center trial \(^{(178)}\). 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 re-stenosis rate in the folate group compared to the placebo group. Repeated target-vessel revascularization was higher in the folate group. In the VISP (The Vitamin Intervention for Stroke Prevention) randomized controlled trials, Toole et al, tested the effect of lowering homocysteine in patients...
with ischemic stroke to prevent stroke, myocardial infarction and death \(^{(179)}\). A total of 3600 adults with non-disabling 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, published the largest metaanalysis of the association of MTHFR 677C→T polymorphism and coronary heart disease \(^{(180)}\). 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, 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 \(^{(181)}\). Systemic arterial compliance increased by 0.15 + 0.03 mL/mmHg \((P=.05)\). These results were independent of homocysteine or folate concentration and MTHFR genotype.

Assanelli et al, 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 \(^{(182)}\).

Finally, the studies by Stott et al, and Nurk et al, published December 2005 are noteworthy \(^{(183-184)}\). Stott et al, studied 185 patients, 65 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\textsubscript{12} (500 mg), vitamin B\textsubscript{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 in cognitive functions at 6 and 12 months. The authors found that while homocysteine levels decreased in the group receiving oral folic acid plus vitamin B\textsubscript{12} supplementation, there was no statistically significant beneficial effects on cognition. Nurk et al, scrutinized the 2,189 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 over time is associated with better cognitive performance.

Silaste et al, reported that a diet high in fresh berries, citrus fruit and vegetables effectively increases serum and RBC folate and decreases plasma homocysteine \textsuperscript{(185)}. Several studies show that Betaine and Choline supplementation lower plasma homocysteine in healthy men and women \textsuperscript{(186-188)}. N-acetylcyesteine therapy is another possible option \textsuperscript{(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. The authors suggest that there are several possible hypotheses relating hyperhomocysteinemia and thrombosis, Table 13. Additional studies are needed to determine which of the six hypotheses is true. Hyperhomocysteinemia is related to atherosclerosis and disorders resulting from arterial vascular disease in a graded manner. This association is modulated by pre-existing 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 (Betaine, 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.
FACTORS THAT INFLUENCE PLASMA HOMOCYSTEINE LEVELS

Table 1  (190)

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 B₆ Deficiency
   a. Inadequate supply
   b. Vitamin B₆ Antagonists: Natural Antagonists and Drug- B₆ 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
FACTORS THAT INFLUENCE PLASMA HOMOCYSTEINE LEVELS

Table 1 (Cont’d)

e. Metformin
f. Disulfide exchangers (D-penicillamine)
g. Gastric Proton Pump Inhibition
h. Vitamin B₆ Antagonists
i. Methyl Group Acceptors (L-Dopa, 6-Mercaptopurine)
j. Other Drugs: (Sulfasalazine, Mega doses 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. Life Style Factors

a. Exercise
b. Smoking
c. Alcohol consumption
d. Coffee intake
e. Vitamin intake
f. Protein intake
FACTORS THAT INFLUENCE PLASMA HOMOCYSTEINE LEVELS

Table 1 (Cont’d)

Genetic

1. Cystathionine – B-synthase deficiency

2. Inborn errors of Folate metabolism
   a. Hereditary folate malabsorption
   b. Methylene tetrahydrofolate Reductase Deficiency (MTHFR).
   c. Glutamate formiminotransferase deficiency

3. Inborn errors of Cobalamin Absorption & Transport
   a. Transcobalamin I (Haptocorrin, R Binder) Deficiency
   d. 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. Methylene tetrahydrofolate Reductase
   b. Methionine Synthase
   c. Methionine Synthase Reductase
Table 2

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 A₂ synthesis
   b. Procoagulant activity: Increased FVIIIc, vWF, thrombin-antithrombin complexes and prothrombin F1&2, and Decreased FVII
   c. Decreased Natural Anticoagulant Activity:
      1. Deficiency of antithrombin
      2. Deficiency of Protein C
<table>
<thead>
<tr>
<th>Classification</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Severe – Moderate Hyperhomocysteinemia</strong></td>
<td>High total homocysteine levels at all times; deficiencies in CBS, MTHFR or in enzymes of B&lt;sub&gt;12&lt;/sub&gt; metabolism.</td>
</tr>
<tr>
<td><strong>Mild – Moderate Hyperhomocysteinemia</strong></td>
<td>Moderately high total homocysteine levels under fasting conditions; reflects impaired homocysteine methylation (folate, B&lt;sub&gt;12&lt;/sub&gt; or moderate enzyme defects, e.g., thermolabile MTHFR).</td>
</tr>
<tr>
<td><strong>Post-methionine load</strong></td>
<td>Abnormal increase in total homocysteine after methionine load. Abnormal net increase reflects impaired homocysteine transsulfuration (heterozygous CBS deficiency, B&lt;sub&gt;6&lt;/sub&gt; deficiency).</td>
</tr>
</tbody>
</table>
Polymorphic Mutations in 5, 10-Methylenetetrahydrofolate Reductase

Table 4

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Change in Amino Acid or Splice Site</th>
<th>Exon or Intron</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>677 CT</td>
<td>Alaine/Valine</td>
<td>Exon 4</td>
<td>20</td>
</tr>
<tr>
<td>1068 TC</td>
<td>Serine/Serine</td>
<td>Exon 6</td>
<td>21</td>
</tr>
<tr>
<td>1178 + 31 T/C</td>
<td>5' Splice Site</td>
<td>Intron 6</td>
<td>22</td>
</tr>
<tr>
<td>1317 T/C</td>
<td>Phenylalanine/Phenylalanine</td>
<td>Exon 7</td>
<td>25</td>
</tr>
<tr>
<td>1298 A/C</td>
<td>Glutamate/alanine</td>
<td>Exon 7</td>
<td>23, 24, 25</td>
</tr>
</tbody>
</table>
### HOMOCYSTEINE EFFECT ON VARIOUS COAGULATION FACTORS

**Table 5**

<table>
<thead>
<tr>
<th>Factor/Process</th>
<th>Effect</th>
<th>Evidence in Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue factor expression</td>
<td>Increase</td>
<td>Suggestive (191)</td>
</tr>
<tr>
<td>FVII activity</td>
<td>Increase</td>
<td>Inconsistent (192-194)</td>
</tr>
<tr>
<td>Thrombin generation</td>
<td>Increase</td>
<td>Suggestive (194-196)</td>
</tr>
<tr>
<td>FV activation</td>
<td>Increase</td>
<td>Suggestive (197, 198)</td>
</tr>
<tr>
<td>Fibrinogen modification</td>
<td>Present</td>
<td>Suggestive (199, 200)</td>
</tr>
<tr>
<td>Thrombomodulin expression</td>
<td>Decrease</td>
<td>Inconsistent (197, 201, 202)</td>
</tr>
<tr>
<td>Inactivation of FVa</td>
<td>Decrease</td>
<td>Inconsistent (203-205)</td>
</tr>
<tr>
<td>TFPI activity</td>
<td>Increase</td>
<td>Inconsistent (206, 207)</td>
</tr>
<tr>
<td>tPA binding</td>
<td>Decrease</td>
<td>Suggestive (208, 209)</td>
</tr>
<tr>
<td>Plasmin generation</td>
<td>Decrease</td>
<td>Suggestive (199, 209, 210)</td>
</tr>
</tbody>
</table>
HYPERHOMOCYSTEINEMIA AND ARTERIAL OCCLUSIVE DISEASE;
STUDIES SHOWING CORRELATION

<table>
<thead>
<tr>
<th>Study</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boushey et al. (120)</td>
<td>5 µmol/L rise in total plasma HC increases relative risk of CAD, CVD, PVD</td>
</tr>
<tr>
<td>European Concerted Action Project (121)</td>
<td>HHC associated with increased risk of vascular disease multiplicative to other risk factors.</td>
</tr>
<tr>
<td>Stampfer et al. (136)</td>
<td>Relative risk of MI of 3.1 when HC levels were in the 95th percentile of control values.</td>
</tr>
<tr>
<td>Malinow et al. (137) &amp; Voutilainen et al. (127)</td>
<td>Increase plasma HC levels are associated with thickened carotid wall</td>
</tr>
<tr>
<td>Nygard et al. (144)</td>
<td>Strong graded relationship between total HC and mortality</td>
</tr>
<tr>
<td>Kluijtmans et al. &amp; Mudd et al. (14-15, 153)</td>
<td>677C→T MTHFR is a genetic risk for CAD</td>
</tr>
</tbody>
</table>

CAD = Coronary Artery Disease
CVD = Cerebrovascular Disease
PVD = Peripheral Vascular Disease
HHC = Hyperhomocysteinemia
HC = Homocysteine
HYPERHOMOCYSTEINEMIA AND ARTERIAL OCCLUSIVE DISEASE;
STUDIES SHOWING NO CORRELATION

Table 7

<table>
<thead>
<tr>
<th>Study</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfthan et al. (146)</td>
<td>No statistical difference between individuals who developed MI and those who did not.</td>
</tr>
<tr>
<td>Verhoef et al. (143)</td>
<td>No statistically significant relative risk to develop CAD, angina and stroke.</td>
</tr>
<tr>
<td>Evans et al. (147)</td>
<td>No association between plasma HC levels and MI.</td>
</tr>
<tr>
<td>Folsom et al. (148)</td>
<td>Total HC levels correlate with CAD in women but not men.</td>
</tr>
<tr>
<td>Brattström et al. (154)</td>
<td>$677C\rightarrow T$ MTHFR is not a causal risk for CAD.</td>
</tr>
</tbody>
</table>

MI = Myocardial Infarction
CAD = Coronary Artery Disease
HC = Homocysteine
<table>
<thead>
<tr>
<th>Study</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Falcon et al. (155)</td>
<td>High prevalence of HHC in juvenile VT</td>
</tr>
<tr>
<td>den Heijer et al. (156-157)</td>
<td>HHC&gt;95(^{th}) percentile of control range is a risk factor for DVT</td>
</tr>
<tr>
<td>Simioni et al. (158)</td>
<td>Significant high prevalence of HHC in patients with DVT of upper extremities</td>
</tr>
<tr>
<td>Eichinger et al. (160)</td>
<td>• HHC in 25% of patients with a single episode of idiopathic VT</td>
</tr>
<tr>
<td></td>
<td>• 2.7 risk of recurrent TE in the first 24 months after discontinuation of anticoagulation</td>
</tr>
<tr>
<td>Kottke-Marchant (126)</td>
<td>Plasma HC &gt; 13 µM is a risk factor for arterial and venous thrombosis in patients with normal coagulation profiles</td>
</tr>
<tr>
<td>Fermo et al. (162)</td>
<td>Moderate HHC in 13.1% of patients with VT and 19.2% of patients with AOD.</td>
</tr>
<tr>
<td>den Heijer et. al. (161)</td>
<td>HHC associated with a calculated pooled odds ratio of 2.6 for VTE</td>
</tr>
<tr>
<td>Arruda et al, Salomon et al, &amp;</td>
<td>Evidence in support of 677→T MTHFR being a risk factor for VT (slightly greater risk for VT in homozygous vs. heterozygous genotype)</td>
</tr>
<tr>
<td>Margaglione et al. (165-167)</td>
<td></td>
</tr>
<tr>
<td>Kluijtmans et al. (173)</td>
<td>677C→T MTHFR may be a risk factor for thrombosis in CBS deficient patients.</td>
</tr>
<tr>
<td>Lalouschek et al. (174)</td>
<td>677C→T MTHFR increased risk of TIA or minor strokes</td>
</tr>
</tbody>
</table>

HHC = Hyperhomocysteinemia
DVT = Deep Venous Thrombosis
HC = Homocysteine
AOD = Arterial Occlusive Disease
VTE = Venus Thromboembolism
VT = Venous Thrombosis
TE = Thromboembolism
HYPERHOMOCYSTEINEMIA AND VENOUS THROMBOSIS;
STUDIES SHOWING NO CORRELATION

Table 9

<table>
<thead>
<tr>
<th>Study</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Martinelli et al. (159)</td>
<td>No association of HHC and DVT of upper extremities</td>
</tr>
<tr>
<td>Trillot et al &amp; Kluijtmans et al. (169, 172)</td>
<td>677C→T MTHFR does not modify risk of VT</td>
</tr>
<tr>
<td>De Stefano et al. (168)</td>
<td>• nine case–control studies involving 2225 patients with VT and 2994 healthy controls</td>
</tr>
<tr>
<td></td>
<td>• No significant differences in cumulative prevalence between homozygous MTHFR in cases with VT vs normal controls</td>
</tr>
</tbody>
</table>

HHC = Hyperhomocysteinemia
VT = Venous thrombosis
Table 10

<table>
<thead>
<tr>
<th>Study</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermo et al. (162)</td>
<td>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.</td>
</tr>
<tr>
<td>Ridker et al. (163)</td>
<td>10 fold increase in thrombotic risk among patients with HHC and FVL.</td>
</tr>
</tbody>
</table>
| Legnani et al. (164)          | • No association between HHC and thrombosis in patients with ptn C, ptn S, AT def or FVL.  
|                               | • $677\text{C} \rightarrow \text{T}$ MTHFR did not confer additional thrombotic risk factor to the heritable thrombophilic coagulation defects |
| Cattaneo et al. (170-171)     | Coexistence of $677\text{C} \rightarrow \text{T}$ MTHFR and FVL increased risk of VT |

VT = Venous Thrombosis  
HHC = Hyperhomocysteinemia  
FVL = Factor V Leiden  
ptn C = Protein C  
ptn S = Protein S  
AT def= Antithrombin deficiency
### NORVIT; EVENT RATES (PER 1000 PERSON-YEARS)

#### Table 11

<table>
<thead>
<tr>
<th></th>
<th>Folic Acid + Vitamin B6</th>
<th>Folic Acid</th>
<th>Vitamin B6</th>
<th>Placebo</th>
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</thead>
<tbody>
<tr>
<td>Primary endpoint</td>
<td>81.6</td>
<td>66.9</td>
<td>70.1</td>
<td>67.2</td>
</tr>
<tr>
<td>MI</td>
<td>73.0</td>
<td>57.5</td>
<td>64.0</td>
<td>59.2</td>
</tr>
<tr>
<td>Death from any cause</td>
<td>37.5</td>
<td>28.7</td>
<td>33.4</td>
<td>31.7</td>
</tr>
<tr>
<td>Cancer</td>
<td>12.0</td>
<td>11.9</td>
<td>8.0</td>
<td>9.0</td>
</tr>
</tbody>
</table>

MI = Myocardial infarction
# NORVIT; RATE RATIOS

## Table 12

<table>
<thead>
<tr>
<th></th>
<th>Folic acid vs control</th>
<th>Vitamin B-6 vs control</th>
<th>Folic Acid + Vitamin B-6 vs control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR 95% CI p</td>
<td>RR 95% CI p</td>
<td>RR 95% CI p</td>
</tr>
<tr>
<td>MI and stroke</td>
<td>1.1 (0.9 – 1.3) .3</td>
<td>1.1 (1.0 – 1.3) .09</td>
<td>1.2 (1.0 – 1.4) .03</td>
</tr>
<tr>
<td>MI</td>
<td>1.1 (0.9 – 1.2) .5</td>
<td>1.1 (1.0 – 1.4) .04</td>
<td>1.2 (1.0 – 1.4) .03</td>
</tr>
<tr>
<td>Death</td>
<td>1.1 (0.9 – 1.3) .8</td>
<td>1.1 (1.0 – 1.5) .11</td>
<td>1.2 (1.0 – 1.5) .10</td>
</tr>
<tr>
<td>Cancer</td>
<td>1.1 (0.9 – 2.0) .08</td>
<td>1.1 (1.0 – 1.4) .30</td>
<td>1.3 (0.8 – 1.9) .30</td>
</tr>
</tbody>
</table>

MI = Myocardial infarction  
RR = Relative Risk  
CI = Confidence interval
HOMOCYSTEINEMIA AND THROMBOSIS

Table 13

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 co-existent risk factors.
6. Hyperhomocysteinemia is a surrogate for low Vitamin-B levels, which is the true risk for vascular disease.
Fig. 1: Schematic representation of homocysteine metabolism

THF: tetrahydrofolate, MTHFR: methylenetetrahydrofolate reductase; MS: methionine synthase; SAM: S-adenosylmethionine, SAH: S-adenosylhomocysteine; BHMT: betaine homocysteine methyltransferase; CBS: Cystathionine-B-synthase; B$_{12}$; B$_{6}$: vitamin B$_{6}$
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1921

1922

1923 210. Harpel PC, Change VT, Borth W. Homocysteine and other sulhydryl compounds enhance the binding of lipoprotein(a) to fibrin: a potential biochemical link between thrombosis, atherogenesis and sulphydryl compound metabolism. Proc. Natl Acad ci USA 1992; 89:10193-10197