



*Advances in Basic, Laboratory and Clinical Aspects of Thromboembolic Diseases**

HYPERHOMOCYSTEINEMIA AND VENOUS THROMBOEMBOLIC DISEASE

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ABSTRACT

Background and Objective. In spite of the large number of reports showing that hyperhomocysteinemia (HHcy) is an independent risk factor for atherosclerosis and arterial occlusive disease, this metabolite of the methionine pathway is measured in relatively few laboratories and its importance is not fully appreciated. Recent data strongly suggest that mild HHcy is also involved in the pathogenesis of venous thromboembolic disease. The aim of this paper is to analyze the most recent advances in this field.

Evidence and Information Sources. The material examined in the present review includes articles and abstracts published in journals covered by the Science Citation Index® and Medline®. In addition the authors of the present article have been working in the field of mild HHcy as cause of venous thromboembolic disease.

State of Art and Perspectives. The studies examined provide very strong evidence supporting the role of moderate HHcy in the development of premature and/or recurrent venous thromboembolic

disease. High plasma homocysteine levels are also a risk factor for deep vein thrombosis in the general population. Folic acid fortification of food has been proposed as a major tool for reducing coronary artery disease mortality in the United States. Vitamin supplementation may also reduce recurrence of venous thromboembolic disease in patients with HHcy. At the present time, however, the clinical efficacy of this approach has not been tested. In addition, the bulk of evidence indicates that fasting total homocysteine determinations can identify up to 50% of the total population of hyperhomocysteinemic subjects. Patients with isolated methionine intolerance may benefit from vitamin B6 supplementation. Homocysteine-lowering vascular disease prevention trials are urgently needed. Such controlled studies, however, should not focus exclusively on fasting homocysteine determinations and folic acid monotherapy.

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Key words: thrombosis, hyperhomocysteinemia, folic acid, vitamin B6, vitamin B12

In spite of the large number of reports showing that hyperhomocysteinemia (HHcy) is an independent risk factor for atherosclerosis and arterial occlusive disease, this metabolite of the methionine pathway is measured in relatively few laboratories and its importance is not fully appreciated. This may be due to a number of reasons. First, diagnosis of HHcy is not straightforward because it requires a combination of fasting and post-methionine load measurement of total plasma homocysteine, which may be cumbersome in outpatients and require determination of local normality ranges. Second, classifying individual patients as being hyperhomocysteinemic has been complicated by recent information that the risk contributed by homocysteine is graded across the distribution of homocysteine values, reproducing a situation similar to that of cholesterol in the era preceding standardization of cholesterol assays. Third, the com-

bined effect on homocysteine levels of vitamin status and genetic abnormalities of the enzymes involved in the methionine pathway is not yet fully appreciated. Last but not least, the high prevalence of HHcy in patients with thrombotic diseases combined with our poor understanding of the specific thrombogenic mechanisms involved underlines the urgent need for identification of a convincing cause-effect relationship.

Recent data strongly suggest that mild HHcy is also involved in the pathogenesis of venous thromboembolic disease. Thus, HHcy and the antiphospholipid antibody syndrome at present represent the only examples of a biochemical abnormality strongly associated with both venous and arterial occlusive disease. Unlike the antiphospholipid antibody syndrome, however, HHcy has the potential to be cured by innocuous vitamin supplementation.

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Methionine metabolism

Homocysteine is a non-protein forming, sulfur amino acid whose metabolism is at the intersection of two metabolic pathways: remethylation and transsulfuration.¹ In remethylation, homocysteine acquires a methyl group to form methionine. In one *de novo* route 5,10-methylenetetrahydrofolate, formed from tetrahydrofolate and serin 3-carbon (PLP-dependent serine hydroxymethyl-transferase, SHMT), is reduced to 5 methyltetrahydrofolate in a physiologically irreversible reaction catalyzed by methylenetetrahydrofolate reductase (MTHFR), an enzyme which contains FAD as a prosthetic group. 5-methyltetrahydrofolate can also be obtained from the circulation since it is the major form of folate in serum and folate-binding proteins or receptors for its internalization are present on most cells. In the next step, the methyl group of 5-methyltetrahydrofolate is transferred to homocysteine in a reaction which is catalyzed by a B₁₂-dependent methyltransferase. The alternative route of homocysteine methylation is through a transfer of a methyl group from betaine, which is catalyzed by a B₁₂ independent methyltransferase (betaine: homocysteine methyltransferase, BHMT). The reaction with N-5-methyltetrahydrofolate occurs in all tissues, while the reaction with betaine is confined mainly to the liver and depends on dietary choline. Most probably due to limited tissue availability,² BHMT is not capable of handling excessive homocysteine accumulation; as a result, in the congenital and acquired defects affecting B₁₂ and the folate-dependent remethylation pathway, the alternative route for conversion of homocysteine to methionine is unable to compensate sufficiently and HHcy results. On the other hand, administration of betaine to homocystinuric patients may improve their clinical condition. A considerable proportion of methionine is then activated by ATP and methionine adenosyltransferase (MAT) to form S-adenosylmethionine (SAM).³ SAM serves primarily as a universal methyl donor to a variety of acceptors, including guanidinoacetate, glycine, nucleic acids, norepinephrine, phosphatidyl-ethanolamine, and hormones. S-adenosylhomocysteine (SAH), the by-product of these methylation reactions, is subsequently hydrolyzed by SAH hydrolase, thus regenerating homocysteine, which then becomes available to start a new cycle of methyl-group transfer.

In the *transsulfuration* pathway, homocysteine condenses with serine to form cystathionine in an irreversible reaction catalyzed by a pyridoxal-5'-phosphate (PLP)-containing enzyme, cystathionine β -synthase (CBS). Human CBS has been cloned recently,⁴ and the gene is located on chromosome 21.⁵ Cystathionine is hydrolyzed by a second PLP-containing enzyme, β -cystathionase, to form cysteine and α -ketobutyrate. Excess cysteine is oxidized to taurine and inorganic sulfates or excreted in the

urine. Thus, in addition to the synthesis of cysteine, this transsulfuration pathway effectively catabolizes excess homocysteine which is not required for methyltransfer and delivers sulfate for the synthesis of heparin, heparan sulfate, dermatan sulfate and chondroitin sulfate.

It is important to note that since homocysteine is not a normal dietary constituent, the sole source of homocysteine is methionine. In mammalian liver approximately half of the methionine entering the methionine cycle undergoes remethylation, while the other half is irreversibly committed to cysteine synthesis through the transsulfuration pathway.⁶ SAM appears to play a key role in regulating the flow of homocysteine towards remethylation or transsulfuration by interacting with CBS, MTHFR and BHMT. When intracellular concentrations of SAM are relatively high, CBS is allosterically activated and homocysteine is diverted to transsulfuration.^{7,8} Conversely, both remethylation pathways are inhibited by SAM.^{9,10} Regulation of methionine metabolism is also affected by tissue levels of individual enzymes, induction of their synthesis by hormones and dietary methionine, as well as by the action of other effector molecules such as SAH acting on BHMT, MTHFR and CBS.¹¹

Due to the existence of a cellular homocysteine export mechanism, plasma normally contains a small amount of homocysteine ($\sim 10 \mu\text{mol/L}$ ¹²). This export mechanism complements the catabolism of homocysteine through transsulfuration; together these mechanisms help maintain low intracellular concentrations of this potentially cytotoxic sulfur amino acid. In HHcy, plasma homocysteine levels are elevated and, barring renal insufficiency,¹³ the occurrence of HHcy indicates that homocysteine metabolism has in some way been disrupted and that the export mechanism is bringing excess homocysteine that has accumulated in the cell into the blood. This limits intracellular toxicity, but leaves vascular tissue exposed to the possibly deleterious effects of excess homocysteine.¹

Pathogenesis of hyperhomocysteinemia

The most severe cases of HHcy are due to homozygous defects in genes encoding for enzymes of homocysteine metabolism. In such cases, a defect of an enzyme involved in either homocysteine remethylation or transsulfuration leads to large elevations of homocysteine in the blood and urine. The classic form of this disorder – homocystinuria – is that caused by a homozygous, or compound heterozygous, defective gene encoding for CBS, a condition in which fasting plasma homocysteine concentrations can be as high as $400 \mu\text{mol/L}$.¹² Depending on the presence of CBS mutants with reduced affinity for the coenzyme,¹⁴ two different forms of the disease can be distinguished on the

basis of responsiveness to treatment with large dosages of pyridoxal-phosphate (vitamin B₆), the CBS cofactor.¹⁵ Several cystathionine β-synthase mutations are known and the most frequent are 833T→C and 919G→A (located in exon 8) and 1224-2A→C, which causes the skipping of the entire exon 12. The 833T→C mutation is present in several ethnic groups; 919G→A has been almost exclusively reported in patients of Celtic origin. In 20 homocystinuric patients from 16 unrelated Italian families, characterization of 24 of 30 independent alleles disclosed 13 mutations, including 11 novel ones. Two previously reported mutations (833T→C and 341C→T) were found in 26.6% and 16.6% of the alleles. Hence most of the mutations are *private* and clustered on exons 8, 3 and 1.^{16,17} Homozygous defects of other genes that lead to similar elevations in plasma homocysteine concentration include those encoding for methylene tetrahydrofolate reductase (MTHFR) or for any of the enzymes which participate in the synthesis of methylated vitamin B₁₂.¹⁸⁻²⁴ Genetic impairments of vitamin B₁₂-dependent methyltetrahydrofolate:homocysteine methyltransferase have not been reported.¹²

MTHFR deficiency was first described by Mudd *et al.*²¹ in two unrelated teen-agers. In contrast to patients with CBS deficiency, these patients had slightly reduced methionine levels in their plasma and normal CBS activity in skin fibroblast extracts. Although the HHcy associated with this defect is less severe than in homozygous CBS deficiency, the prognosis for these patients is generally poorer than in CBS deficiency,²⁵ due at least in part to unresponsiveness to any form of treatment in MTHFR deficiency. Most patients with MTHFR deficiency have hypomethioninemia, but in contrast to patients whose hypomethioninemia is due to inborn errors of vitamin B₁₂ metabolism and who develop severe megaloblastic anemia,²⁶ patients with MTHFR deficiency do not become anemic.

Expression of MTHFR deficiency is however variable. Mutations that result in severely reduced MTHFR enzyme activity are rare and associated with HHcy and clinical manifestations of varying severity.²⁷⁻²⁹ However, recent evidence indicates a prevalent variant of MTHFR that demonstrates reduced activity. In 1988, Kang *et al.*²⁴ reported that two unrelated patients with moderate HHcy and low folate levels had a variant MTHFR that is distinguished from the normal enzyme (as measured in lymphocyte extracts) by its lower specific activity (50%) and its thermolability. In subsequent studies, Kang *et al.*^{30,31} demonstrated that MTHFR thermolability is an inherited recessive trait which is present in approximately 5% of the general population and 17% of patients with proven coronary artery disease, but is not associated with neurological complications. Impaired MTHFR activity due to the thermolabile form of the enzyme has been observed

in as many as 28% of hyperhomocysteinemic patients with premature vascular disease.³² The cDNA for human MTHFR has recently been isolated²⁸ and it has been shown that MTHFR thermolability is caused by a point mutation (677C to T transition) at a polymorphic site, resulting in a valine substitution for an alanine in this enzyme.³³ The mutation was found in 38% of unselected chromosomes from 57 French-Canadian individuals; the homozygous state of the mutation was present in 12% of these subjects and correlated with significantly raised tHcy.³³ Preliminary evidence indicates that the frequency of homozygotes for the 677C→T mutation may vary significantly in populations from different geographic areas (from 1.4% to 15%, 34). In a Dutch population, homozygosity for this mutation was 15% and 5% in 60 vascular patients and 111 controls, respectively.³⁵ In 289 Italian controls and 64 patients with arterial or venous occlusive disease, we found a prevalence of homozygosity of 16.2% in controls, of 62.1% in patients with moderate fasting HHcy, and 2.8% in the remaining patients with normal fasting HHcy.³⁶ Interestingly, none of 7 patients with isolated methionine intolerance was homozygous for the mutation.³⁷

The impact of the MTHFR thermolabile variant on plasma HHcy is as yet unclear. The HHcy seen in Kang *et al.*'s original patients²⁴ was associated with low folate plasma levels, and folate supplementation reduced homocysteine to normal levels. In a recent study an interaction between the MTHFR thermolability genotype and folate status was demonstrated.³⁸ When plasma folate concentrations were > 15.4 nmol/L, plasma homocysteine levels were low and unrelated to the MTHFR genotype. However, when plasma folate concentrations were < 15.4 nmol/L, plasma homocysteine levels were significantly higher in homozygotes for the *ala* to *val* mutation than in those with the normal genotype.³⁸ Since MTHFR is part of the remethylation pathway, HHcy caused by homozygosity of the *ala* to *val* mutation will be manifested under fasting conditions and not after a methionine load. These data imply that phenotypic expression of the MTHFR genotypes is dependent on the availability of folate, suggesting that homozygotes for the thermolabile genotype might have a higher folate requirement than individuals with a normal genotype. Interestingly, the presence of the MTHFR thermolabile mutation was not found to be a risk factor for coronary artery disease in a large Australian population.³⁹

Because plasma homocysteine determinations were not carried out in this study, this finding does not rule out HHcy as a risk factor for coronary artery disease, but it does rather suggest vitamin status as a major determinant of plasma homocysteine levels, even in the presence of mild enzymatic defects of methionine metabolism. Since FAD is an

essential prosthetic group for MTHFR activity, it stands to reason that vitamin B₂ status is also a determinant of plasma homocysteine levels.

The interrelationship between genetic defects of the enzymes of the methionine metabolic pathway, nutritional status and the expression of HHcy is complex and undergoes fine tuning.^{40,41} Even mild vitamin deficiencies may be responsible for moderate HHcy.

Plasma homocysteine concentrations in these instances may differ depending on which arm of the two metabolic pathways of homocysteine metabolism is defective.¹ An impairment in the remethylation pathway, even if it is mild, will lead to a substantial increase in plasma homocysteine concentrations under fasting conditions. This impairment may be due to inadequate status of folate or vitamin B₁₂ or B₂, or to defects in the gene encoding for MTHFR.^{1,24,42-55} In contrast, a mild impairment in the transsulfuration pathway will lead, at most, to a very slight increase in fasting plasma homocysteine levels. This mild impairment, which may be due to heterozygous defects in the CBS gene or inadequate levels of vitamin B₆,^{1,42,56-60} is normally identified by an abnormal increase in plasma homocysteine after a methionine loading test or following a meal.^{42,60-63} The different phenotypes expected in remethylation and transsulfuration defects are supported by studies conducted in vitamin deficient animal models. Thus, fasting plasma homocysteine concentration is 10-fold higher in folate deficient rats than in folate supplemented rats.⁶⁴ This high concentration of homocysteine in plasma was due in part to lack of sufficient S-adenosylmethionine for the activation of the transsulfuration pathway.⁶⁴ In both humans and rats, mild vitamin B₆ deficiency was associated with normal fasting plasma homocysteine levels. Fasting HHcy in vitamin B₆ deficiency occurs only if the deficiency is severe and sustained over a long period of time.⁵⁸ After a methionine load, the homocysteine concentration increased 35-fold in rats that were vitamin B₆ deficient, compared to about 4-fold in control rats and less than 35% in folate deficient rats.⁶⁴

Evidence of two distinct forms of HHcy in humans derives from preliminary data obtained in participants in the Framingham Family Heart Study.⁶⁵ For each of 274 participants, plasma homocysteine concentrations were measured at fasting and 4 hours after a methionine load. Using the 90% percentile values to define HHcy and considering the post-methionine load (PML) change in homocysteine from baseline levels, equal proportions of the participants had either fasting or PML HHcy without the other, whereas only 12% had both.⁶⁵

Assays of plasma homocysteine

Homocysteine circulates in plasma as free homocysteine and as the homocysteinyl moiety of the disulfides homocystine and cysteine-homocysteine, both free and bound to protein. The concentration of free reduced homocysteine is very low and accounts for less than 5% of total plasma homocysteine in normal subjects.⁶⁶ Hence in the assessment of HHcy, it is important that all plasma forms of homocysteine be measured.

A variety of assay methods have been described, with *normality* ranges which are slightly, but significantly, different (reviewed in refs. #67, 68). Homocysteine levels are different in plasma and serum, in women and men, and there is an increase in homocysteine levels with increasing age.^{69,70} These differences may be related to variations in vitamin status and should be kept in mind in the identification of hyperhomocysteinemic individuals.

Confusion about the dependency of HHcy – particularly of mild to moderate degree HHcy – on vitamin status is largely due to non-standardization of preanalytical conditions. *Baseline* and *fasting* homocysteine levels are not synonymous; whereas fasting homocysteine is correlated in both patients and controls with the levels of vitamin B₁₂ and folate and to a much lesser – if any – degree to pyridoxal phosphate (vitamin B₆) levels, the same may not be true for so-called *baseline* homocysteine. Oral methionine loading (usually 0.1 g/kg body weight) is used to detect heterozygotes for CBS deficiency, who usually but not always⁷¹ respond with abnormally high elevations in post-load homocysteine levels. Plasma levels of methionine and of reduced free homocysteine reach a peak within 2 hours,⁷² while homocysteine is highest only after 6 to 8 hours. Neither methionine clearance nor post-load total homocysteine is affected by excess dietary methionine in normal individuals.⁷³

Post-methionine load homocysteine levels have been measured between 4 and 8 hours after methionine intake in different studies, which may have implications in the reported prevalence of abnormalities of homocysteine transsulfuration. Even more importantly, post-load homocysteine determinations should be expressed as the net difference over fasting levels for accurate detection of HHcy resulting from defective transsulfuration.

To facilitate expanded application of the methionine loading test, a shortened 2-hour protocol was recently validated.⁷⁴

The 2-hour plasma homocysteine level accounted for > 92% of the variability in the 4-hour plasma homocysteine level.⁷⁴ The 2-hour loading test may offer distinct advantages in terms of participant acceptability and logistical considerations in epidemiologic and clinical settings.

Hyperhomocysteinemia and venous thromboembolic disease

Although venous thromboembolism accounts for 50% of the vascular complications of homocystinuria,⁷⁵ the potential for involvement of less severe HHcy in the pathogenesis of venous thromboembolic disease had been overlooked until recently. The expression of inherited biochemical abnormalities predisposing to venous thromboembolic consists of recurrent thrombosis, thrombosis at a young age, idiopathic thrombosis, thrombosis after trivial provocation, and thrombosis in an unusual site.⁷⁶ Brattstrom *et al.*⁷⁷ found a higher prevalence of HHcy after a methionine load (14%) – but not of fasting HHcy – in a series of patients under 50 years of age with venous thromboembolism than in sex- and age-matched controls. Increased fasting homocysteine levels were reported in another study in 25% of patients who developed venous thrombosis before 60 years of age.⁷⁸ Fasting and post-methionine load homocysteine levels were measured by Falcon *et al.*⁷⁹ in a series of 80 patients who had had at least one verified episode of venous thromboembolism before the age of 40 years and who were free from hemostatic abnormalities known to be associated with increased risk of venous thromboembolism. Fasting HHcy was observed in 8.8% of patients, but post-methionine load HHcy was present in 17.7% of them. About half of the patients with HHcy had a positive family history of thrombosis and familial HHcy was confirmed in over 50% of the families studied. In a cross-sectional 2-year evaluation of 157 consecutive unrelated patients with a history of venous or arterial occlusive disease occurring before the age of 45 years or at unusual sites, moderate HHcy was detected in 13.1% and 19.2% of patients with venous or arterial occlusive disease, respectively.⁸⁰ The prevalence of HHcy was almost twice as high when based on homocysteine measurements made after oral methionine load as when based on fasting levels. Deficiencies of protein C, protein S, plasminogen and activated protein C resistance were detected only in patients with venous occlusive disease, with an overall prevalence of 18.7%. Familial HHcy was demonstrated in 8 of the 12 families investigated. Event-free survival analysis showed that the relative risk in patients with moderate HHcy and the other defects was 1.7 times greater than in patients without defects and that the risk conferred by HHcy was similar to that of defects affecting the protein C system. A higher rate of recurrent thrombosis was also observed in patients with HHcy and with the other defects than in patients without defects.⁸⁰ Homocysteine levels above the 90th percentile of the control distribution were observed in another study in 25% of 185 patients with recurrent venous thrombosis, with a relative risk of recurrence 2 times greater in patients with HHcy than in those

without it.⁸¹ In this study, the relative risk of patients with post-methionine load homocysteine concentrations exceeding the 90th percentile (2.6) was similar to that of patients with fasting HHcy. Twenty-seven of the 46 patients with fasting HHcy also had post-load HHcy, whereas 17 patients had isolated methionine intolerance. Hence the overall prevalence of HHcy in this patient population was 34.1%. However, since absolute post-methionine load values (instead of the post-methionine load changes from baseline levels, 65) were considered in this study, the relative contribution of remethylation or transsulfuration defects to the risk conferred by HHcy cannot be extrapolated.⁸²

These data represent very strong evidence supporting the role of moderate HHcy in the development of premature and/or recurrent venous thromboembolic disease. High plasma homocysteine levels are also a risk factor for deep vein thrombosis in the general population. Fasting homocysteine concentrations were measured in 269 patients under 70 years of age with a first episode of deep-vein thrombosis and matched control subjects participating in the Leiden Thrombophilia Study.⁸³ HHcy exceeding the 95th percentile of the control group was found in 10% of the patients, with a matched odds ratio of 2.5. The effect of HHcy was independent of other well-established risk factors for thrombosis, including protein C, protein S and antithrombin III deficiencies and activated protein C resistance. An unexpected finding of this study was the observation that the association between elevated homocysteine levels and venous thrombosis was stronger among women than among men. Unlike the above mentioned studies on patients with venous thromboembolism which excluded from the analysis subjects with reduced folate and vitamin B₁₂ levels, nutrient levels were not measured in this study. Thus, it cannot be ruled out that the stronger association observed in women may depend on a different micronutrient status. In addition, post-methionine load homocysteine measurements were not carried out, resulting in a potential underestimation of the risk conferred by HHcy.

Similar to deficiencies of the protein C anticoagulant system, not all patients with HHcy develop thrombosis. The possibility that contributory factors in addition to HHcy may be required for the development of thrombotic manifestations was explored in 45 members of seven unrelated consanguineous kindreds in which at least one member was homozygous for homocystinuria.⁸⁴ Thrombosis occurred before the age of 8 years in 6 of 11 patients with homocystinuria; all six patients also showed activated protein C resistance. Conversely, of four patients with homocystinuria who did not have activated protein C resistance, none experienced thrombosis before the age of 17 years. The authors concluded for a substantially increased risk

of thrombosis in patients with both homocystinuria and activated protein C resistance.⁸⁴ This conclusion may cast doubts about an independent pathogenic role for HHcy in venous thromboembolism. Both activated protein C resistance and HHcy are highly prevalent in patients with early onset vascular occlusive disease.⁸⁰ If the association of APC-resistance and moderate HHcy markedly increased the thrombotic risk, one would expect its prevalence to be significantly higher than what is associated with the prevalence of the isolated defects. In a series of 307 patients with early-onset venous or arterial disease or with thrombosis occurring at unusual sites, the prevalence of isolated APC-resistance and moderate HHcy (fasting or post-methionine load) were 10% and 27%, respectively. The combined defect was detected in 3.6% of patients, a figure slightly, but not significantly higher than the 2.7% prevalence expected assuming no effect of the association on the risk of thrombosis.⁸⁵ Notwithstanding the fact that identification of a laboratory abnormality of thrombophilia should obviously not prevent a search for other hereditary thrombotic disorders, it should be concluded that *isolated* moderate HHcy is an independent risk factor for both venous and arterial thromboembolic disease.

Thrombogenic mechanisms of hyperhomocystinemia

A number of arguments directly implicate circulating homocysteine levels as an etiologic factor for thrombosis. Some evidence stems from deficiencies of folate, vitamin B₁₂ and B₆, which cause an increase in homocysteine levels. Inflammatory bowel diseases, which lead to folate malabsorption, are known to be associated with an abnormal incidence of thrombotic disease. Folate deficiency is also present in myeloproliferative disorders, which predispose to both arterial and venous thrombotic episodes. Additional hints are provided by drugs which affect vitamin absorption and/or metabolism and which are associated with both HHcy and an increased incidence of thrombotic episodes. Methotrexate is a folate antagonist. It has been proposed that the increased incidence of thromboembolism seen in patients receiving methotrexate may be related to the observed elevation in plasma homocysteine levels.⁸⁶ There is evidence that long-term use of oral contraceptives is associated with folate deficiency.⁸⁷ Estrogen-containing contraceptives also affect pyridoxine metabolism and may influence homocysteine levels through the transulfuration mechanism.⁸⁸ Azauridine, a drug used in the treatment of psoriasis, inhibits CBS activity,⁸⁹ giving rise to HHcy; the use of this drug has also been associated with vascular occlusive complications. The absence of a strong association between

nutrient deficiencies of folate, vitamin B₁₂ and B₆ and thromboembolic complications is most probably linked to the issue of disease duration.¹² In subjects with genetic abnormalities of methionine metabolism, normal or mildly reduced nutrient levels may give rise to long-lasting HHcy of variable severity. Conversely, for severe vitamin deficiencies in the absence of inherited defects of the methionine metabolic pathway, by the time homocysteine levels rise to the range of moderate to severe HHcy, the other clinical features associated with the deficiency state lead the patient to seek medical attention, with correction of the underlying nutrient deficiency. However, 56 of 115 patients with pernicious anemia died of cerebrovascular accidents, cardiac failure and coronary thrombosis.⁹⁰ Mild to moderate HHcy occurs in patients with chronic renal disease,^{13,91-93} particularly in patients on chronic hemodialysis and in spite of vitamin supplementation.⁹³ HHcy may be a contributory factor to the high prevalence of vascular disease in patients with chronic renal insufficiency.⁹⁴ On the other hand, a remarkable absence of atherosclerosis and thromboembolic disease has been observed in patients with trisomy 21 (Down's syndrome), who have approximately 150% normal CBS activity.⁹⁵ The protective effect of CBS gene dosage has been reported in 3 of 4 reported studies.⁹⁶⁻⁹⁹

A number of investigators have tried to elucidate the thrombogenic mechanism(s) of HHcy. Early animal studies suggested a toxic effect of HHcy on endothelial cells, resulting in shortened platelet survival,^{72,100} but these data have not received confirmation.^{101,102}

In vitro studies of cultured endothelial cells also showed a toxic effect of homocysteine on cell viability and function, but these studies were conducted using extremely high homocysteine concentrations (1-10 mmol), exceeding the levels encountered even under the most severe pathological conditions.^{100,103-105} Non-specific inhibition of prostacyclin synthesis¹⁰⁶ and activation of factor V¹⁰⁷ by high concentrations of homocysteine on cultured endothelial cells has been reported. Inhibition of protein C activation¹⁰⁸ and downregulation of thrombomodulin expression¹⁰⁹ at homocysteine concentrations > 5 mmol/L have also been observed. One to five mmol homocysteine specifically block t-PA, but not plasminogen binding to endothelial cells.¹¹⁰ The toxic effect of high homocysteine concentrations on endothelial cells¹¹¹ also results in increased platelet adhesion¹⁰⁶ because of impaired regulation of endothelium-derived relaxing factor and related nitrogen oxides,¹¹² induction of tissue factor,¹¹³ suppression of heparan sulfate expression,¹¹⁴ and stimulation of smooth muscle cell proliferation.¹¹⁵ HHcy induces oxidation of low-density lipoprotein *in vitro*.¹¹⁶ Since homocysteine can participate in disulfide bond exchange reactions, it is possible that excessive homocysteine entering the

circulation can alter plasma proteins by this process. It has been reported that homocysteine concentrations as low as 8 mmol/L dramatically increased the affinity of Lp(a) for plasmin-modified fibrin surfaces, thus inhibiting plasminogen activation.¹¹⁷

It is generally held that different mechanisms are responsible for arterial and venous thromboembolic diseases, involving platelet function abnormalities in arterial thrombosis and abnormalities of coagulation and/or fibrinolysis in venous thromboembolism. *Ex vivo* studies looking for such abnormalities in patients with HHcy have yielded inconclusive results.^{101,102,118-123} In subjects with severe HHcy due to a homozygous CBS deficiency, abnormally high *in vivo* biosynthesis of thromboxane A₂ – as reflected by urinary excretion of its major metabolite 11-dehydro-thromboxane B₂ – has been observed.¹²⁴ Administration of aspirin inhibited thromboxane production with return to baseline high levels with a time course consistent with platelet survival, suggesting platelets were a major source of increased thromboxane urinary excretion.¹²⁴ Because thrombin is a potent inducer of platelet activation, the presence of a hypercoagulable state was investigated in homocystinuric patients.¹²⁵ Increased circulating levels of prothrombin fragment 1.2, thrombin-antithrombin complex and activated protein C were observed in homocystinuric subjects essentially free of vascular disease. Interestingly, protein C levels, which were reduced to a greater extent than factor VII and factor II levels, were significantly correlated with the degree of HHcy.¹²⁵ Diet-responsive deficiency of factor VII was previously reported in CBS-deficient patients.^{122,123,126} Reduced protein C levels may contribute at least in part to the venous thrombotic manifestations of patients with homozygous CBS deficiency. These observations may have an impact on the treatment of HHcy because increased urinary thromboxane excretion was independent of homocysteine levels and was present both in vitamin B₆ responsive and non-responsive patients.¹²⁵ It is noteworthy that although the effectiveness of vitamin B₆ in preventing thromboembolism in pyridoxine-responsive patients was shown to be highly statistically significant, the occurrence of thromboembolism was not abolished by vitamin supplementation.⁷⁵

Conclusions

Folic acid fortification of food has been proposed as a major tool for reducing coronary artery disease mortality in the United States.¹²⁷ Vitamin supplementation may also reduce the recurrence of venous thromboembolic disease in patients with HHcy. At the present time, however, the clinical efficacy of this approach has not been tested. In

addition, the bulk of evidence indicates that fasting total homocysteine determinations can identify up to 50% of the total population of hyperhomocysteinemic subjects. Patients with isolated methionine intolerance may benefit from vitamin B₆ supplementation. Homocysteine-lowering vascular disease prevention trials are urgently needed. These controlled studies should not, however, focus exclusively on fasting homocysteine determinations and folic acid monotherapy.

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