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Hyperhomocysteinemia and thrombosis

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Since the discovery that homocystinuria is associated with vascular thrombotic disease in children [1,2], much research has been directed at understanding the relation of homocysteine to arterial and venous thrombotic disease in adults [3]. A great deal of this research effort has been directed toward answering two questions: Is homocysteine itself responsible for, or simply a marker of, increased thrombotic potential? Can dietary and lifestyle changes and vitamin supplements reduce the thrombotic risk associated with hyperhomocysteinemia? This article reviews homocysteine metabolism, the causes of hyperhomocysteinemia, possible mechanisms of increased atherogenesis and thrombosis, and the clinical evidence linking hyperhomocysteinemia with venous and arterial thromboses.

Homocysteine metabolism

Remethylation and transsulfuration

Homocysteine is an intermediary in the methionine cycle (Fig. 1). Methionine derived from dietary sources is first converted into S-adenosylmethionine (SAM). This important intermediate serves as the key methyl donor for a large number of biochemical reactions. After donating a methyl group, SAM is converted to S-adenosylhomocysteine, which undergoes hydrolysis to form adenosine and homocysteine. Homocysteine is remethylated to form methionine by one of two pathways. The major pathway is remethylation by methionine synthase (MS) in a reaction that requires vitamin B_{12} as a cofactor (in the form of methylcobalamin) and converts N^5-methyltetrahydrofolate (5-methylTHF) to

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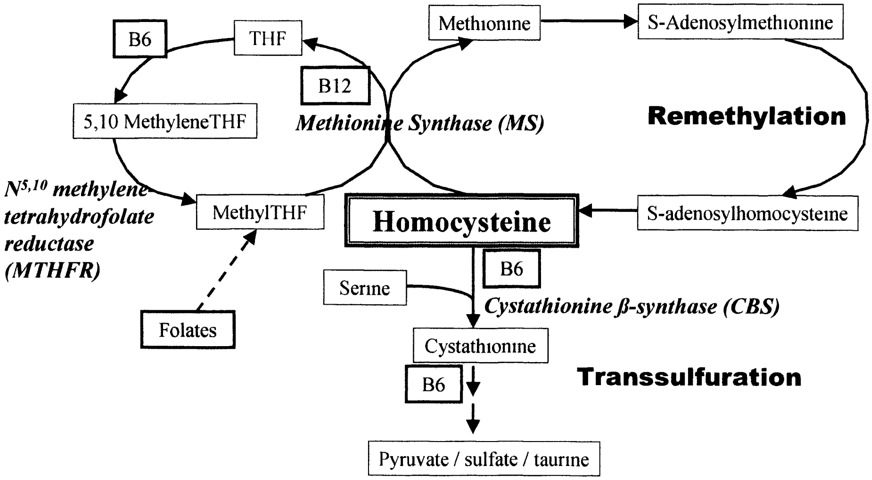

Fig. 1. Methionine cycle and homocysteine metabolism. Homocysteine is an intermediary in the methionine cycle. Most homocysteine is remethylated to regenerate methionine by the action of methionine synthase. This enzyme requires methyl-tetrahydrofolate (methyl-THF) as a cosubstrate and vitamin B_{12} as a cofactor. Methyl-THF is regenerated in a multistep process, with the final step catalyzed by N [5,10] methyleneTHF reductase (MTHFR). Collectively, these 2 cycles form the remethylation pathway of homocysteine metabolism. The other pathway is the transsulfuration pathway initiated by CBS. Homocysteine metabolized by this process generates smaller molecules that are excreted by the kidney. Three key enzymes in which mutations and polymorphisms have been discovered are shown in italics *(MS, CBS, MTHFR).* Steps where vitamins are required are shown together with the vitamin in the shaded box.

tetrahydrofolate (THF). THF is recycled into MTHF in a two-step process; the first step converts THF to N [5],N^{10}-methylenetetrahydrofolate, and the second converts N [5],N^{10}-methylenetetrahydrofolate back to 5-methylTHF. This second step is catalyzed by methyltetrahydrofolate reductase, an enzyme under much study because of a common mutation that produces mild hyperhomocysteinemia. The minor homocysteine remethylation pathway that uses betaine as a methyl donor becomes important clinically in the treatment of patients with homocystinuria [4]. The methionine cycle and the linked 5-methylTHF regeneration cycle form the primary pathway of homocysteine metabolism known as the remethylation pathway.

Alternatively, homocysteine can be metabolized by the transsulfuration pathway. Homocysteine can condense with serine to form cystathionine by the action of cystathionine β-synthase (CBS). A congenital deficiency of this enzyme causes most of the homocystinuria cases associated with early-onset atherosclerosis. Pyridoxal phosphate (vitamin B_6) serves as a cofactor in this reaction and in the subsequent reaction that converts cystathionine to cysteine and α-ketobutyrate. Cysteine is eventually metabolized into taurine and sulfates that are excreted by the kidneys.

Hyperhomocysteinemia: definitions and classification

Homocysteine exists in various forms within the body (Fig. 2). Chemically, *homocysteine* (Hcy) refers to the reduced form of the molecule that is normally present in small amounts [5]. More commonly, Hcy is found in an oxidized form, either combined with another Hcy through a disulfide link to form *homocystine* or with other sulfhydryl groups to form mixed disulfides. Collectively, Hcy, homocystine, and the mixed disulfides form a fraction termed free homocysteine (fHcy). The other major fraction is bound homocysteine (bHcy) linked through disulfide bridges to plasma proteins, primarily albumin. Most clinical studies report total plasma homocysteine (tHcy), measured after treatment with reducing agents to liberate both fHcy and bHcy from their disulfide linkages. Whether specific homocysteine species are responsible for accelerated atherogenesis is unknown, but some believe the reduced form has greater potential for harm [6]. In this chapter, hyperhomocysteinemia refers to elevated levels of tHcy measured in the fasting state, and homocystinuria refers to the group of several inborn errors of metabolism causing markedly elevated serum tHcy with resultant loss of Hcy in the urine.

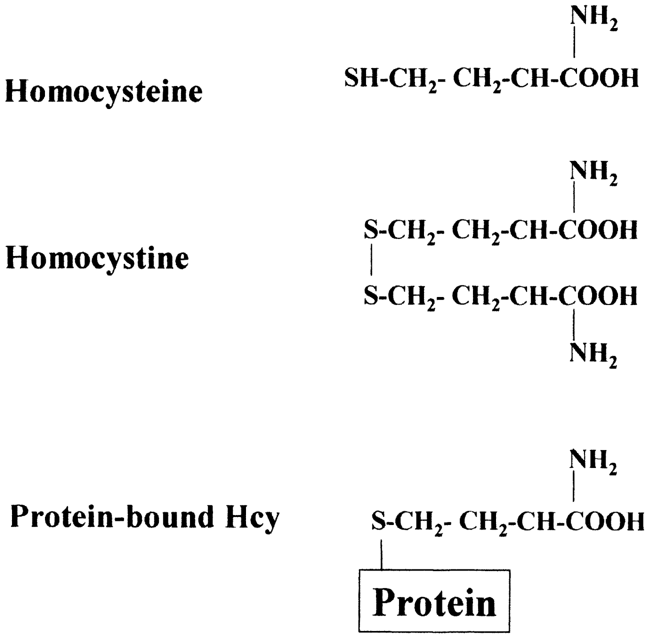

Fig. 2. Structures of homocysteine moieties. Chemically, homocysteine refers to the reduced form of the molecule, which is normally present in low concentrations within the body. Most homocysteine is found either in the oxidized form (ie, joined with another homocysteine molecule to form homocystine) or is bound to a variety of plasma proteins. Treatment to free-protein–bound and oxidized homocysteine moieties allows total plasma homocysteine to be measured.

There is not universal agreement on the definition of hyperhomocysteinemia. Kang et al [7] proposed using cutoffs to separate normal (5–15 μmol/L) from mild (15–30 μmol/L), moderate (30–100 μmol/L), and severe (greater than 100 μmol/L) hyperhomocysteinemia. Because factors such as age, gender, diet, smoking, and genetic influences all have impacts on tHcy levels, however, no single definition has been established for hyperhomocysteinemia. As such, any definition will be arbitrary and will not reflect that the vascular risk associated with tHcy is a continuum that increases directly with the tHcy level [8].

In addition to tHcy values, homocysteine measured after oral methionine loading is under investigation as a tool to identify persons at greater risk for vascular disease. The methionine-loading test (MLT) involves two measurements, a baseline tHcy and a repeat value 2 to 6 hours after ingestion of a standard quantity of methionine (usually 100 mg/kg body weight). Similar to fasting tHcy, there is no standard definition of an abnormal post-MLT tHcy value. An abnormal result is thought to represent mild abnormalities in the transsulfuration pathway in which the fasting tHcy level is normal but rises abnormally under conditions in which the transsulfuration pathway is stressed (methionine excess, pyridoxal phosphate deficiency) [9]. This is a more cumbersome test to perform, and its clinical usefulness remains to be seen.

Causes of hyperhomocysteinemia

Major mutations in homocysteine metabolism: homocystinuria

The intense scrutiny of homocysteine was ignited by observations that venous and arterial occlusive disease developed in patients with homocystinuria. Homocystinuria is caused by a variety of enzymatic defects; the predominant one is CBS deficiency. Patients with CBS deficiency can have tHcy levels higher than 400 μmol/L [10]. In descending order of frequency, venous thromboembolism, cerebrovascular events, peripheral arterial disease, and myocardial infarction were reported in a large survey of CBS-deficient patients worldwide [11]. Autopsy specimens often revealed arterial and venous thrombi with damage to the vessel wall [12].

Minor mutations in homocysteine metabolism

MTHFR mutations: Cys677Thr and Ala1298Cys

Mutations that severely reduce MTHFR activity are rare. In 1988, Kang et al [13] reported a thermolabile mutation of MTHFR associated with less specific enzyme activity and hyperhomocysteinemia. In 1994 the *MTHFR* gene was cloned, and, shortly thereafter, Frosst et al [14] identified the common polymorphism of MTHFR (Cys677Thr) that showed thermolability and produced hyperhomocysteinemia. This point mutation replaces an alanine with a valine residue in the enzyme.

It is now known that the Cys677Thr mutant allele occurs at high frequency in Europe (up to 50%) [15], variable frequency in Asia (approximately 45% in Koreans, 30% in Chinese) [16,17], lower frequency in the United States (approximately 30% in whites, 10% in African Americans, and 35% in Hispanics) [18], and rarely in Africa [15]. Given the high T-allele frequency in many populations, heterozygosity and homozygosity are common. It has been consistently shown that heterozygotes have no significant levels of hyperhomocysteinemia, and homozygotes have levels of hyperhomocysteinemia that are most apparent in those who are folate deficient [19,20]. A large meta-analysis has shown that the TT genotype raises tHcy values by an average of 25% [21]. Because of its frequency in the general population (approximately 10%–12%), the 677TT genotype contributes a significant amount to hyperhomocysteinemia. The TT genotype has been found in 30% to 50% of patients with hyperhomocysteinemia with fasting levels above the 90th percentile [21] and in 75% with fasting levels above 40 μmol/L [22,23]. A remarkable feature of this enzymatic defect is that it can be ameliorated by oral vitamin supplementation, as will be discussed.

A second MTHFR polymorphism, Ala1298Cys, leads to a glutamine-for-alanine exchange. The presence of the 1298Cys allele lowers MTHFR enzyme activity but, interestingly, does not alter tHcy values, even in the homozygous state [24]. So far, no clinical association has been shown between this mutation and vascular disease [25,26], as would be expected given that Hcy levels are not altered by this polymorphism.

Methionine synthase and cystathionine β-synthase mutations

Interest in methionine synthase polymorphisms grew out of the observation that an Ala2756Gly polymorphism was relatively common in the general population [27]. Subsequent studies have shown that carriers of the G allele (in either heterozygous or homozygous form) have neither hyperhomocysteinemia nor increased risk for cardiovascular, cerebrovascular, or venous thrombotic disease [28–30].

There are several known cystathionine β-synthase mutations, including the two that usually cause homocystinuria in homozygous form, Thr833Cys and Gly919Ala. Heterozygotes for these mutations occur in less than 1% of the population [11] and lead to elevated fasting homocysteine values [31]. Given the relative scarcity of these enzyme mutations, however, they account for only a small fraction of the patients with hyperhomocysteinemia. A more common mutation, found in 10% of the general population, is a 68-bp insertion in exon 8 of the *CBS* gene. This polymorphism, termed 844ins68, is actually associated with slightly *lower* tHcy values after MLT but has no effect on fasting tHcy levels [29,32]. Allele frequencies of the insertion variant were no different between controls and those with arterial or venous thrombotic diseases [30,33,34]. Although the insertion polymorphism has a neutral effect on homocysteine levels, two recent studies suggest that its pairing with the MTHFR 677TT genotype leads to increased thrombotic risk [23,35].

Physiological/lifestyle

Plasma tHcy levels increase with age [36,37], and men have higher levels than women [38,39]. In women, elevated estrogen levels are correlated with lower tHcy levels: premenopausal women have lower tHcy levels than postmenopausal women even after adjustment for age [40,41]. Postmenopausal women taking hormone replacement therapy have lower tHcy levels than those not taking estrogenic compounds [42], and pregnant women have lower tHcy levels than their nonpregnant counterparts [43,44].

The effect of alcohol on homocysteine levels is uncertain [38,45], but coffee drinking [46] and smoking [38,47] consistently increase tHcy levels in large population-based studies. As seen in Fig. 3, the effects of individual lifestyle and physiological changes appear mild, and significant overlap exists among all the variables. The combined effects of multiple factors, however, may cause a substantial impact on tHcy levels (ie, older men who smoke and drink coffee).

Vitamin deficiency

Vitamins play a key role in the remethylation and transsulfuration pathways of homocysteine metabolism (Fig. 1). Active forms of vitamin B_{12} (methylcobalamin, a cofactor for methionine synthase), folate (5-methylTHF, a cosubstrate for

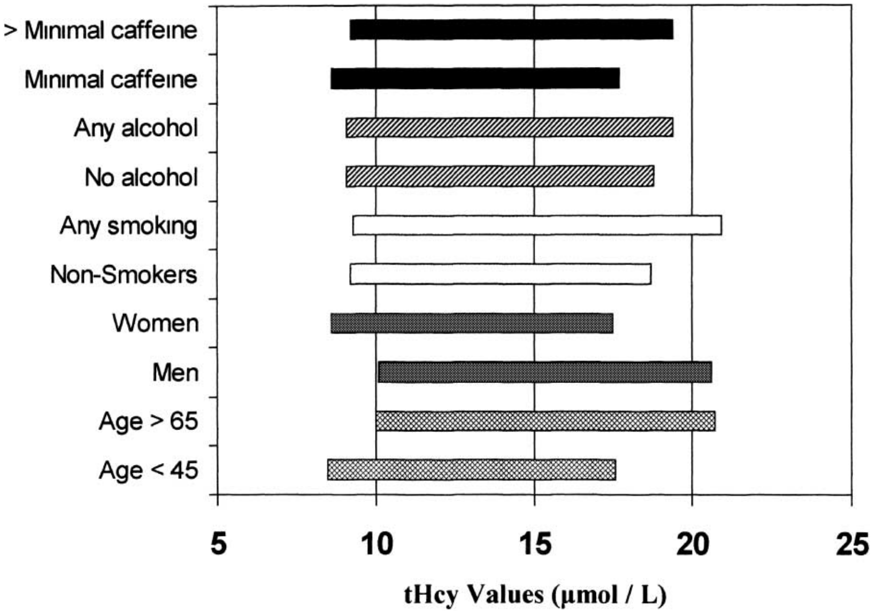

Fig. 3. Effects of lifestyle on homocysteine levels. Caffeine and alcohol intake, smoking, gender, and age can all affect total homocysteine (tHcy) values. (*From* Jacques PF, Bostom AG, Wilson PW, et al. Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. Am J Clin Nutr 2001;73:613–21; with permission.)

methionine synthase), vitamin B_6 (cofactors for three enzymes, including CBS), and riboflavin (roles in vitamin B_6 and MTHFR activation) all must be present in sufficient amounts for proper regulation and control of the methionine cycle and homocysteine levels (Fig. 4).

Vitamin B_{12} (cobalamin) deficiency is associated with hyperhomocysteinemia [48,49]. Even in persons without vitamin B_{12} deficiency there is an inverse relationship between serum B_{12} and tHcy levels [50]. Furthermore, treatment with vitamin B_{12} has been shown to lower homocysteine values in persons with and without B_{12} deficiency [51,52]. Similar findings have been shown for vitamin B_6 [53].

Folate deficiency is also associated with hyperhomocysteinemia [54], an association first described in 1987. Subsequent clinical trials evaluating the effect of folate supplementation on tHcy levels were summarized in a meta-analysis [55]. The authors concluded that folate in therapeutic doses (0.4 mg and above daily) lowers tHcy levels by approximately 25% in patients with normal and elevated pretreatment tHcy levels. Although there was not a significant dose–response relationship with folate, patients with higher pretreatment tHcy levels seem to derive the most benefit. Additional supplementation with vitamin B_{12} produced further lowering of tHcy.

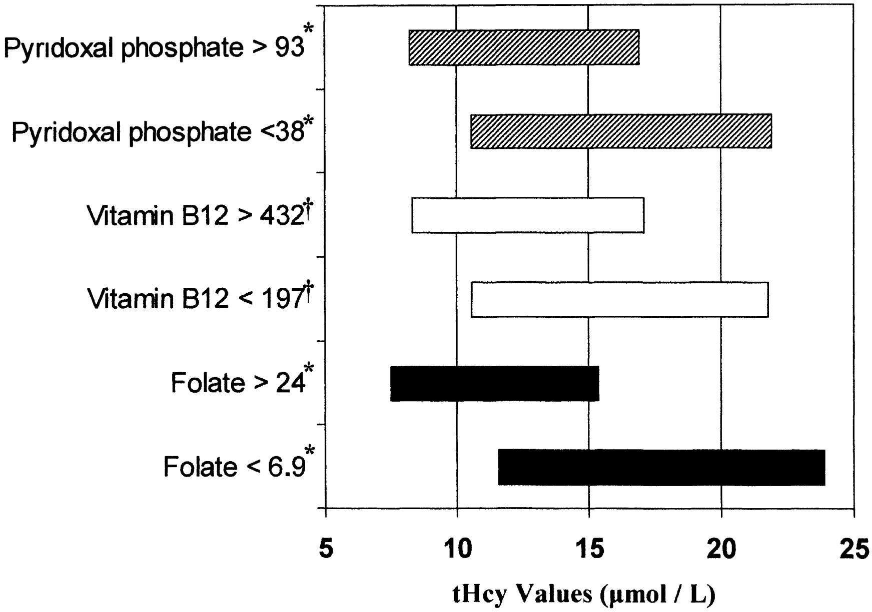

Fig. 4. Effects of plasma vitamin levels on homocysteine levels. Deficiencies of pyridoxal phosphate, vitamin B_{12}, and folate are all associated with increased homocysteine concentrations. * Pyridoxal phosphate and folate in nmol/L; †vitamin B_{12} in pmol/L. (*From* Jacques PF, Bostom AG, Wilson PW, et al. Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. Am J Clin Nutr 2001;73:613–621; with permission.)

Renal insufficiency

Renal insufficiency is associated with hyperhomocysteinemia, and the degree of homocysteine elevation is inversely related to the creatinine clearance [56,57]. Levels in patients on chronic dialysis average approximately 30 to 35 μmol/L [58,59]. The mechanism is unclear but may be caused by a remethylation defect in uremia given that the kidney itself does not actively participate in homocysteine clearance or metabolism [60]. Treatment with vitamin B_{12} [61] and folate [62,63] lowers tHcy levels in these patients.

Hyperhomocysteinemia and vascular thrombosis

Venous thromboembolism

Taken in aggregate, studies evaluating hyperhomocysteinemia as a risk factor for venous thromboembolism (VTE) have shown a positive correlation. Two recent meta-analyses published in 1998 [64,65] pooled data from 9 different studies and included more than 800 VTE patients. The two studies found an overall odds ratio (RR) of 2.5 to 2.9 for the association of hyperhomocysteinemia and VTE. Hyperhomocysteinemia was defined as levels above the 95th percentile of the control population, and similar odds ratios were found whether fasting tHcy or post-MLT tHcy levels were used. It seems reasonable to conclude that hyperhomocysteinemia is a risk factor for VTE, but more recent data have produced some puzzling findings that challenge this conclusion.

Because the MTHFR Cys677Thr polymorphism is common in the general population and patients with 677TT display hyperhomocysteinemia, TT homozygotes should be overrepresented in VTE populations if hyperhomocysteinemia is a risk factor. In a review of 9 case–control studies (more than 2000 patients with venous thrombosis) by de Stefano et al [66], however, the prevalence of the TT genotype was equal in the thrombosis populations (13.9%) compared with the control populations (13.7%). The absence of any association of MTHFR 677TT with VTE casts some doubt as to the exact relation between homocysteine level and venous thrombosis risk.

Additional studies have examined the interaction between hyperhomocysteinemia and the MTHFR Cys677Thr polymorphism to other hypercoagulable states. In particular, interactions with factor V Leiden (FV Leiden) have been studied because of its high prevalence in the general population and its clear association with VTE [67–70]. Most of the studies have failed to show an interaction, but the study by Ridker et al [71]— the only prospective study—is notable for finding an additive VTE risk for hyperhomocysteinemia with FV Leiden (adjusted relative risk [RR] = 9.7 for both versus RR = 2.1 for FV Leiden alone). Because only 4 patients in the VTE group had tHcy levels greater than the 95th percentile and were FV Leiden positive, no definitive conclusions can be drawn. Similarly, most studies assessing interactions between MTHFR 677TT and FV Leiden have shown no additive risk above the risk with FV Leiden alone

[25,72–77]. No additional risk in TT homozygotes with the prothrombin Gly20210Ala mutation has been reported [66].

Cerebrovascular disease

The realm of arterial thrombotic disease (cardiovascular and cerebrovascular diseases) has been studied extensively in relation to hyperhomocysteinemia. A review of 17 studies that evaluated homocysteine in stroke patients was published by Moller et al [78]. Eight cross-sectional and 4 longitudinal studies were included in the final analysis, and the authors calculated an odds ratio of 4.12 (cross-sectional) and 3.74 (longitudinal studies) for stroke when tHcy values exceeded the 95th percentile of the control populations. A subsequent cohort study suggested a linear relation between tHcy levels and risk for stroke in the elderly [79]. As with VTE, the association between hyperhomocysteinemia and stroke seems strong but, unlike the VTE data, is bolstered by several prospective studies that come to conclusions similar to those for the case-control studies.

When the MTHFR Cys677Thr polymorphism is examined in stroke patients, no clear relationship is found. Wu et al [80] combined data from 7 stroke studies in which the MTHFR Cys677Thr polymorphism was examined. More than 1300 stroke patients and 1700 control patients were included, and there was no overall increase in the odds ratio for stroke in patients with the TT genotype. More recent studies not included in this analysis have also failed to find an association [81,82].

The data for stroke patients parallels the data for VTE patients. Despite an apparent association between hyperhomocysteinemia and vascular thrombosis (VTE or stroke), a common mutation that clearly causes hyperhomocysteinemia is not overrepresented in the study population. This again raises the possibility that the observed link of high tHcy levels to vascular thrombosis is not causal, as suggested by some investigators [83].

Coronary artery disease

The data for coronary artery disease (CAD) and hyperhomocysteinemia is the most extensive, yet controversy exists as to how the data are best interpreted. An early review in 1995 by Boushey et al [8] concluded that a 5-μmol/L increase in homocysteine levels was associated with an odds ratio of 1.6 for the development of CAD in men and 1.8 in women. The authors estimated that 10% of coronary artery disease in the population as a whole is attributable to hyperhomocysteinemia. In their analysis, 17 studies were included, including 2 prospective studies. More recently, 2 other reviews have been published to reexamine the link using newer data. In the meta-analysis by Cleophas [84], 33 studies were evaluated. The overall odds ratio for CAD was 1.58 and highly significant ($P < 0.001$), but the authors suggested that homocysteine may be a marker for unhealthy lifestyles rather than a risk factor for CAD. When the data from prospective studies (OR, 1.49) was separated from the others (OR, 1.62), the link was less strong, suggesting that bias inherent in retrospective studies may have skewed the data in favor of a link. Furthermore, 5 of the 11 prospective studies reviewed did not show a significant link between hyperhomocysteinemia and CAD. The review by Christen et al [85], covering 43 studies, also found that prospective data showed a weak to nonexistent link of tHcy values to CAD. A major concern is that homocysteine levels may rise after tissue damage [86]; thus, retrospective studies of CAD patients may show elevated tHcy values as a result of, not a cause of, the coronary events.

Ambiguous results extend to studies addressing the influence of MTHFR Cys677Thr on CAD. Brattstrom et al [21] published a review of the Cys677Thr polymorphism and its effect on homocysteine levels and vascular disease. Twenty-three studies and nearly 6000 patients with all types of vascular disease (but predominantly CAD) were included in the analysis. No difference between vascular patients and controls was found for the frequency of the T allele (34% in both groups) or the TT genotype (12% in both groups). Wu et al [80] published a similar analysis but separated those studies that exclusively examined CAD. More than 2000 patients were analyzed, but, unlike the Brattstrom [21] result, an overall link with the TT genotype was suggested (OR = 1.3; 95% confidence interval [CI], 1.11–1.52). A closer look at the data shows that the positive correlation of the TT genotype came from only 3 of the 10 studies, focused only on Japanese patients with CAD. Whether this ethnic disparity exists in the role of MTHFR Cys677Thr in CAD remains to be seen.

Overall, no link between the MTHFR Cys677Thr polymorphism and CAD can be established, at least in white populations. There is a stronger association between homocysteine levels and CAD, but the disparity between retrospective and prospective studies suggests a noncausal link between the two conditions.

Homocysteine and enhanced thrombosis: possible mechanisms

Nitric oxide inhibition and vascular endothelial dysfunction

Direct endothelial toxicity of homocysteine may result from oxidative stress. Cytotoxicity results when human endothelial cells are exposed to supraphysiological levels of homocysteine, a process that can be blocked by treatment with catalase [87].

Aside from being a potent vasodilator, nitric oxide released from endothelial cells inhibits platelet activation, adhesion, and aggregation and may have other beneficial effects [88]. Homocysteine interacts with nitric oxide on many levels. First, homocysteine can combine with nitric oxide to form S-nitroso-homocysteine, a molecule that has vasodilatory and antiplatelet effects and may be one way that excess homocysteine is detoxified [89]. Second, high homocysteine levels indirectly impair the synthesis of nitric oxide through the inhibition of nitric oxide synthase [90]. Third, high homocysteine levels impair endothelium-dependent vasodilation [91,92]. Recent trials with Hcy-lowering vitamin treatment show improved vasodilation after treatment [93,94].

Protein homocysteinylation

Homocysteine, though structurally similar to other amino acids, is normally not incorporated into proteins. Nevertheless, the insertion of Hcy into a growing polypeptide chain can occur, and it does so at a rate that is proportional to homocysteine concentrations [95] and lysine content [96] of the polypeptide. Experimentally, enzymes can become inactive if homocysteine residues are incorporated [96]. In addition, homocysteine itself or a product from protein editing, homocysteine thiolactone, may modify proteins after translation [97,98]. In the former, homocysteine has been shown to incorporate into factor V at cysteine residues, causing functional activated protein C resistance in patients with hyperhomocysteinemia [97].

Interaction with clotting and fibrinolytic factors

In vitro studies have shown that the incubation of cultured endothelial cells with high levels of homocysteine may cause activation of the clotting system and inhibition of the fibrinolytic system. Enhanced tissue factor activity [99], activation of factor V [100], down-regulation of thrombomodulin [101,102], factor Va resistance to activated protein C [97], and impaired tissue plasminogen activator binding to endothelial cells [103] have all been reported. Whether one major mechanism or a combination of mechanisms is clinically relevant awaits further investigation.

Treatment of hyperhomocysteinemia

Fortunately, treatment of hyperhomocysteinemia is a less controversial topic. Many factors can raise Hcy levels, as described above. Although some cannot be modified, there is evidence that lifestyle changes can lower Hcy levels [46].

Most intervention studies have focused on the ability of vitamin supplementation to lower homocysteine. Because folate, vitamin B_{12}, and vitamin B_6 all have important roles in homocysteine metabolism, these have been the most studied. Individually, supplementation with folate [104,105], vitamin B_{12} [51,61], or vitamin B_6 [106] can be shown to decrease homocysteine levels, but most studies have used a combination of vitamins. Folate shows the strongest lowering effect (25%), with some added benefit of vitamins B_6 (8%) and B_{12} (7%) [55,106]. Although there is some debate as to exact dosing required to achieve maximal effect, 1 mg folate is effective [107]; higher doses do not appear to add further benefit [55].

Additional findings from these trials are clinically important:

- First, the Hcy-lowering effect of vitamin intervention is most pronounced in patients with the highest baseline Hcy values [55]. Data from the Homocysteine Lowering Trialists' Collaboration showed that patients in the lowest quintile of baseline Hcy had a 16% relative reduction, whereas those in the highest quintile had a 39% reduction, even after adjustment for folate dosing. Thus, those with the highest pretreatment Hcy levels gain the greatest benefit with vitamins.

- Second, vitamin supplementation improves homocysteine levels regardless of the specific disease state. Patients with VTE [105] and CAD [93,108] have similar responses to folate. In addition, MTHFR 677TT patients also respond well to folate [109,110], though they may require higher doses [22,59,110]. Patients with chronic renal insufficiency [62] or end-stage renal disease [59,111] also respond to vitamin supplementation, despite the fact that dialysis patients do not normalize their values.

Summary

Homocysteine remains an enigmatic marker for vascular disease. Studies have shown hyperhomocysteinemia is a risk factor for VTE, cerebrovascular disease, and coronary artery disease. This relationship, however, has not been consistently corroborated by studies of patients with genetic polymorphisms that alter homocysteine metabolism. Studies at the molecular level reveal interactions between homocysteine, the endothelium, and the clotting system. Further investigation at the basic science level is needed to determine whether homocysteine is a marker of vascular injury and thrombotic potential or whether it plays a pathogenic role.

Preliminary trials with vitamins clearly show that safe, inexpensive treatment can lower homocysteine levels. The clinical impact on decreasing vascular disease, however, has yet to be shown. Until there is evidence that treatment improves outcomes, testing for homocysteine and treating hyperhomocysteine-

Table 1
Ongoing clinical trials assessing benefit of vitamins in vascular disease

Study	Sample size	Comments
Vitamin Intervention for Stroke Prevention (VISP)	3,600	High vs low-dose folate + B6 + B12
Women's Antioxidant and Cardiovascular Disease Study (WACS)	8,000	Folate + B6 + B12 vs placebo
Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH)	12,000	Folate + B12 vs placebo and simvastatin 20 mg vs 80 mg (2 × 2 design)
Cambridge Heart Antioxidant Study (CHAOS-2)	4,000	Folate vs placebo
Norwegian Study of Homocysteine Lowering with B Vitamins in Myocardial Infarction (NORVIT)	3,000	Folate + B12 vs placebo and B6 vs placebo (2 × 2 design)
Western Norway B Vitamin Trial (WENBIT)	2,000	Folate + B12 vs placebo and B6 vs placebo (2 × 2 design)
Prevention with a Combined Inhibitor and Folate in Coronary Heart Disease (PACIFIC)	10,000	Folate vs placebo with omapatrilat (2 × 2 design)
Heart Outcomes Prevention Evaluation-2 Study (HOPE-2)	5,000	Folate + B6 + B12 vs placebo

mia will be a debatable issue. A series of vitamin intervention trials begun in 1997 will enroll tens of thousands of patients (Table 1) and will, it is hoped, provide the necessary information for developing evidence-based guidelines.

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