Editorial

Over 25 years ago, McCully reported that elevated plasma homocyst(e)ine concentrations can cause atherosclerotic vascular disease (1). His postmortem analysis of several patients with elevated plasma homocyst(e)ine due to rare inborn errors of metabolism showed severe atherosclerosis. Abundant epidemiologic evidence has since confirmed McCully's original hypothesis, with elevated plasma homocyst(e)ine concentrations conferring an independent, increased risk for coronary, cerebrovascular, and peripheral vascular atherosclerotic disease (2, 3).

Homocysteine is a sulfur-containing amino acid formed during the metabolism of methionine. Once synthesized, homocysteine may undergo remethylation to methionine in a reaction catalyzed by methylenetetrahydrofolate homocysteine methyltransferase (methionine synthetase), which uses methyltetrahydrofolate as a methyl donor and cobalamin as an essential cofactor (4). Alternatively, homocysteine can enter the transsulfuration pathway when cysteine synthesis is required or in the presence of excess methionine. In this pathway, homocysteine first condenses with serine to form cystathionine in a rate-limiting reaction catalyzed by cystathionine-β-synthase and requiring pyridoxal 5′-phosphate; cystathionine-γ-lyase then catalyzes the hydrolysis of cystathionine to yield α-ketobutyrate and cysteine in another reaction requiring pyridoxal 5′-phosphate.

Several genetic disorders have been associated with hyperhomocyst(e)inemia, including a deficiency of cystathionine-β-synthase, a mutation that confers thermolability to 5, 10-methylenetetrahydrofolate reductase, and a deficiency of methylenetetrahydrofolate homocysteine methyltransferase. Cystathionine-β-synthase deficiency, an autosomal recessive trait, is the most common identifiable cause of hyperhomocyst(e)inemia. Individuals homozygous for a deficiency of this enzyme are rare (1:200,000 births) and develop atherothrombotic vascular disease in childhood and adolescence that is frequently fatal (5).

In the general population, hyperhomocyst(e)inemia has proven to be much more common than originally believed, with an estimated prevalence of 1:70. This high prevalence was appreciated only recently owing to the development of a methionine challenge test (6). Among patients with cerebrovascular, coronary, or peripheral atherosclerosis, hyperhomocyst(e)inemia is found in up to 40% of individuals (7).

More recent data also suggest that deficiencies of vitamin cofactors required for homocysteine metabolism, namely folate, pyridoxine, and cobalamin, can promote hyperhomocyst(e)inemic atherothrombosis. Selhub and colleagues have argued that most individuals with increased plasma homocyst(e)ine concentrations have inadequate concentrations of one or more of these vitamins (8), an observation supported by the recent study of Robinson and colleagues with respect to pyridoxal 5′-phosphate (9). Vitamin supplementation has been shown to reduce or normalize elevated plasma homocyst(e)ine levels (10, 11), although the benefits of vitamin therapy with respect to clinical end points have not yet been prospectively evaluated.

In this context, the report by Ubbink and colleagues (12) in this edition of *The Journal* extends the initial observations on the role of pyridoxine in homocysteine metabolism in an interesting and cleverly designed clinical study. These investigators examined the effects of a selective deficiency of pyridoxal 5′-phosphate on plasma homocyst(e)ine levels after an oral methionine challenge. This deficiency occurred in asthmatic individuals chronically treated with theophylline, a competitive inhibitor of pyridoxal kinase (13). They observed in these individuals with normal folate and vitamin B-12 concentrations that theophylline treatment led to significantly higher plasma homocyst(e)ine and serum cystathionine concentrations after methionine challenge compared with controls. 6 wk of pyridoxine supplementation improved these abnormal responses to methionine challenge, presumably by effectively restoring plasma concentrations of pyridoxal sufficient to compete effectively with plasma theophylline concentrations. This observation provides the first compelling evidence that subnormal concentrations of pyridoxal 5′-phosphate alone can induce methionine intolerance, an independent risk factor for homocyst(e)ine-induced atherothrombotic disease. In view of the recent report that smokers have significantly lower plasma pyridoxal 5′-phosphate concentrations than nonsmokers (14), results of this study have potential implications for a large segment of the population of patients at risk for atherosclerosis, and, perhaps, even more so for those smokers with reactive airways disease treated with methylxanthines.

In another related report in this edition of *The Journal*, Lentz and co-workers (15) examined the effects of hyperhomocyst(e)inemia on vascular function in a primate model. Both clinical observations (5) and animal studies (16) clearly indicate the adverse consequences of hyperhomocyst(e)inemia on vascular structure; however, the effects of elevated levels of plasma homocyst(e)ine on endothelial function and vascular responses have not heretofore been investigated in an in vivo model without underlying vascular disease. These investigators demonstrated that diet-induced hyperhomocyst(e)inemia led to blunted responses of resistance vessels to endothelium-dependent vasodilators, and that this effect was accompanied by depressed thrombomodulin activity and moderately reduced vascular smooth muscle responses to nitrovasodilators when vessels were studied ex vivo. These data support the implications of the study by Celermajer and colleagues (17), who demonstrated impaired endothelium-dependent vasodilator function in homozygous hyperhomocyst(e)inemics, and the study by Van den Berg and co-workers (18), who demonstrated abnormal endothelial antithrombotic function in young, mildly hyperhomocyst(e)inemic individuals with peripheral arterial occlusive disease. In both of these studies, however, the investigators could not distinguish between homocysteine and frank atherosclerosis as the basis for endothelial dysfunction. The data by Lentz and colleagues, by contrast, provide the first compelling evidence for the pathophysiologic

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effects of hyperhomocyst(e)inemia in vivo in the absence of structural vascular disease.

It is instructive to consider in more detail the potential molecular and cellular mechanisms by which hyperhomocyst(e)inemia evokes abnormal vascular responses, both from an historic perspective and in light of more recent experimental data. Homocysteine can be viewed as the first risk factor for atherosclerosis believed to exert its effects through a mechanism involving oxidative damage (19, 20). Homocysteine is readily oxidized when added to plasma, principally as a consequence of auto-oxidation leading to the formation of homocystine, homocysteine-mixed disulfides, and homocysteine thiolactone. During oxidation of the sulfhydryl group, superoxide anion radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) are generated, and these oxygen-derived molecules are believed to account for the endothelial cytotoxicity of homocyst(e)ine. That oxidant stress is induced in vivo by hyperhomocyst(e)inemia is indicated by the decrease in plasma cysteine with an accompanying increase in plasma cystine concentrations (21). $O_2^{\cdot-}$ and hydroxyl radical ($OH^{\cdot}$) generated during oxidation of homocysteine initiate lipid peroxidation, an effect that occurs both at the endothelial cell surface as well as within lipoprotein particles in plasma (22). Heinecke and colleagues have suggested that cystine is necessary for the production of $O_2^{\cdot-}$ by vascular cells and their ability to oxidize low-density lipoprotein (23).

Upon exposure to homocyst(e)ine, endothelial dysfunction occurs at several levels. The normal antithrombotic endothelium is converted to a more prothrombotic phenotype with increased Factor V and Factor XII activity, decreased protein C activation with accompanying inhibition of thrombomodulin expression, induction of tissue factor expression, and suppression of heparan sulfate expression. Homocyst(e)ine also reduces the binding of tissue-type plasminogen activator to its endothelial cell receptor, annexin II. All of these changes have as their final common action facilitation of the generation of thrombin.

The vasodilator properties of the normal endothelial cell are also altered by homocyst(e)ine, specifically with respect to endothelial nitric oxide ($NO^{\cdot}$) production. We have shown previously that normal endothelial cells detoxify homocysteine by releasing nitric oxide or a related *S*-nitrosothiol which, in turn, leads to the formation of *S*-nitroso-homocysteine (24). With exposure to homocysteine, endothelial cells first respond by increasing their production of *S*-nitrosothiols, including *S*-nitroso-homocysteine. In addition, this increase in *S*-nitroso-homocysteine appears to be accompanied by an increase in steady state *Nos3* mRNA levels in the endothelial cell. *S*-Nitrosation of homocysteine attenuates its pathogenicity by inhibiting sulfhydryl-dependent generation of H_2O_2; in addition, as with other *S*-nitrosothiols, *S*-nitroso-homocysteine is a potent vasodilator and platelet inhibitor. This protective action of $NO^{\cdot}$, however, is eventually overcome by chronic exposure of the endothelial cell to hyperhomocyst(e)inemia. Increasingly compromised production of $NO^{\cdot}$ ultimately leads to unopposed homocyst(e)ine-mediated oxidative injury to the endothelium.

Sulfhydryl compounds, such as homocysteine, are believed to attenuate endothelial production of bioactive nitric oxide through the reaction of $O_2^{\cdot-}$ generated during their auto-oxidation (25) with $NO^{\cdot}$, resulting in the formation of the adduct peroxynitrite ($OONO^-$). Thus, despite the increased production of $NO^{\cdot}$ after homocysteine exposure, less bioactive $NO^{\cdot}$ is available owing to its inactivation by $O_2^{\cdot-}$ produced during homocysteine's oxidation. This mechanism likely accounts for the results of Lentz and colleagues reported here in which endothelium-dependent vasodilator responses were attenuated following homocyst(e)ine exposure in a primate model (15).

In addition to the protection, albeit weak, afforded the endothelium by enhanced $NO^{\cdot}$ production in response to homocyst(e)ine exposure, other protective mechanisms exist in the vasculature to attenuate the oxidative injury evoked by this amino acid. Although thiol auto-oxidation generates $O_2^{\cdot-}$, H_2O_2, and $OH^{\cdot}$, it is primarily H_2O_2 (and derived $OH^{\cdot}$) that has been implicated in the vascular toxicity of hyperhomocyst(e)inemia. Once formed, H_2O_2 freely crosses cell membranes and is efficiently reduced to water by catalase or by glutathione peroxidases. While the former is quite catalytically efficient, its K_m for H_2O_2 is two to three orders of magnitude weaker than that for the glutathione peroxidases. In addition and of importance, catalase cannot reduce lipid peroxides but the glutathione peroxidases can.

The antioxidant enzyme family, the glutathione peroxidases, catalyzes the reduction of both hydrogen and lipid peroxides to their corresponding alcohols in a reaction mechanism that involves the oxidation of glutathione. Three isoforms of the enzyme have been identified thus far, an erythrocytic isoform, as well as intracellular and extracellular isoforms, both of which are selenocysteine-containing proteins. We have shown recently that by reducing these peroxides to alcohols, glutathione peroxidase prevents inactivation of $NO^{\cdot}$ (26). We have also shown recently that homocyst(e)ine both inhibits glutathione peroxidase activity in vitro and leads to a dramatic reduction in steady state mRNA levels for the intracellular isoform in endothelial cells; importantly, these effects occur at concentrations of homocysteine that are pathophysiologically relevant and are not produced by cysteine (27). That the inhibition of glutathione peroxidase is unique to homocysteine compared with other biologic thiols may provide an explanation for the unique vascular toxicity of homocysteine and the lack of toxicity of cysteine, an amino acid present in plasma at a concentration at least three- to fourfold greater than that of homocysteine and equally capable of generating $O_2^{\cdot-}$ during auto-oxidation. In conjunction with this reduction in intracellular glutathione peroxidase message and enzyme activity, homocysteine also significantly decreases the thiol redox state of vascular cells, as measured by the ratio [GSH]/[GSSG], through oxidation of GSH (28); in so doing, a relative deficiency of cosubstrate for glutathione peroxidase develops, further impairing endothelial oxidative defense mechanisms and potentiating peroxyl radical–mediated $NO^{\cdot}$ inactivation with resulting endothelial dysfunction. Recently we identified a family with a deficiency of extracellular glutathione peroxidase, which was accompanied by a dramatic impairment of $NO^{\cdot}$-mediated platelet inhibition and a thrombotic disorder (26).

As one final measure of the effects of homocyst(e)ine-induced endothelial dysfunction, it is useful to consider the effects of endothelial-derived $NO^{\cdot}$ on vascular smooth muscle proliferation. Normal endothelial $NO^{\cdot}$ impairs underlying vascular smooth muscle cell migration and proliferation; however, homocyst(e)ine itself also leads to vascular smooth muscle cell proliferation in vitro, in part by a mechanism involving increased cyclin D_1 and cyclin A mRNA expression (29). This effect may, in part, account for the abnormal response to exoge-

nous nitrovasodilators in the study by Lentz and colleagues (15); alternatively, one can argue that the oxidative stress imparted by hyperhomocyst(e)inemia can inactivate exogenous nitrovasodilators equally well as it inactivates endothelial $NO^{\cdot}$.

Hyperhomocyst(e)inemia has evolved from a unique, relatively rare mechanism for atherothrombosis to a remarkably common disorder in the general atherosclerotic population. Its association with oxidative stress in the vasculature, with vitamin deficiency states, and with endothelial dysfunction all serve to cast this interesting disorder in terms consonant with a contemporary understanding of the complex pathogenesis of atherothrombotic vascular disease.

Joseph Loscalzo
Whitaker Cardiovascular Institute
Evans Department of Medicine
Boston University School of Medicine
Center for Advanced Biomedical Research

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