Oxidative stress due to the production of intracellular and extracellular reactive oxygen species may be a major player in the pathogenesis of cardiovascular and other diseases. Because homocysteine and other thiols have pro-oxidant activity, the oxidant stress hypothesis is frequently invoked to explain the damaging effects of homocysteine on vascular cells and tissues. However, the underlying mechanisms of homocysteine-induced vascular injury are still largely unknown in subjects with elevated plasma total homocysteine (tHcy) levels. It is now well established that hyperhomocysteinemia is an independent risk factor for coronary artery disease, cerebrovascular disease, and peripheral vascular occlusive disease.3–5 Yet the question remains: Is homocysteine causal or merely an innocent marker? Several recent studies suggest that mild hyperhomocysteinemia, either basal or transient after a methionine load, can impair endothelial cell function.6–11 Because antioxidants were effective in blocking endothelial dysfunction during transient hyperhomocysteinemia, it was suggested that oxidative stress was involved in the mechanism.12-14 Additional support for the oxidative stress hypothesis has come from in vitro studies of cultured endothelial and smooth muscle cells. However, many of these studies used supraphysiological concentrations (1 to 10 mmol/L) of homocysteine under conditions that, in all likelihood, led to the generation of reactive oxygen species in the absence of in vivo antioxidant defense systems.

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Homocysteine contains a reactive sulfhydryl group (-SH) and, like most thiols (RSH), can undergo oxidation to the disulfide (RSSR) at physiological pH in the presence of O2:

\[
2 \text{RSH + O}_2 \rightarrow \text{RSSR + [O}_2^-] \rightarrow \text{H}_2\text{O}_2
\]

The general reaction is catalyzed by transition metals.15 and a variety of reactive oxygen species can be produced, including superoxide anion radical and hydrogen peroxide.16,17 Examination of the forms of homocysteine in circulation support the concept of thiol oxidation in vivo. Greater than 98% of tHcy in normal (5 to 12 μmol/L) and mildly hyperhomocysteinemic (>12 to 25 μmol/L) plasma is oxidized, consisting of the disulfide dimer homocystine (5% to 10%), the mixed disulfide with cysteine and other thiols (5% to 10%), and mixed disulfides with protein (≥80%). Only in severe hyperhomocysteinemia, in which tHcy ranges from 50 to 500 μmol/L, is there evidence that free, reduced homocysteine composes a substantial portion (up to 20%) of tHcy.18

The daily flux of plasma homocysteine in a healthy 70-kg individual is estimated to be ~1.3 mmol/d.19 Let us assume that all of the homocysteine entering the circulation is reduced and that it undergoes rapid oxidation to homocystine and mixed disulfides with cysteine and protein. This being the case, ~0.65 mmol/d of hydrogen peroxide would be generated as a result of homocysteine oxidation, which is equivalent to 92 pmol · min⁻¹ · mL⁻¹ blood in a 70-kg individual (assuming an approximate blood volume of 70 mL/kg). Someone with mild hyperhomocysteinemia (eg, tHcy=20 μmol/L) would generate twice the level of hydrogen peroxide (184 pmol · min⁻¹ · mL⁻¹) under the same set of assumptions. The transient hyperhomocysteinemia observed 4 to 8 hours after methionine loading6–11 and 12 hours after consumption of an animal protein meal11 may also contribute to hydrogen peroxide flux. However, the putative “oxidative stress” generated by homocysteine oxidation in mild hyperhomocysteinemia and the transient hyperhomocysteinemia associated with methionine loading pales in comparison with the potential oxidative stress due to cysteine flux.

The concentration of total cysteine in normal plasma is 25 to 30 times higher than that of tHcy, and oxidized forms of cysteine (cystine and mixed disulfides) account for 94% to 95% of total cysteine.20 Generating oxidative stress through the daily flux of cysteine21 would appear to be much greater than that for homocysteine, yet cysteine is not usually considered to be a significant risk factor of cardiovascular disease (however, see Reference 22). Moreover, the blood is not without a potent built-in antioxidant defense system that includes low-molecular-weight antioxidants (ascorbate, tocopherols, etc), glutathione peroxidase, superoxide dismutase (SOD), and catalase.

If homocysteine-induced oxidant stress is responsible for vascular injury and dysfunction, it is likely to occur in subjects with classic homocystinuria, an autosomal recessively inherited disorder affecting homocysteine metabolism. The most common form of homocystinuria, affecting from 1:200 000 to 1:50 000 newborns, is due to cystathionine β-synthase (CBS) deficiency. This pyridoxal 5'-phosphate–dependent enzyme diverts homocysteine from the methionine cycle into the transsulfuration pathway. Approximately 50% of the patients with CBS deficiency respond to pyridoxine therapy, resulting in a dramatic decrease in tHcy, in some
cases to normal levels. Pyridoxine nonresponders are aggressively treated with folate acid, vitamin B₁₂, pyridoxine, betaine, and low-methionine diets, resulting in substantial decreases of tHcy levels. However, normalization of tHcy is rarely achieved in nonresponders, and these individuals do remarkably well with few or no cardiovascular events, in spite of the fact that their tHcy may range from 50 to 100 μmol/L.

In this issue, Wilcken et al²⁴ showed that extracellular SOD (EC-SOD), a Cu,Zn isofrom of SOD, was highly correlated with tHcy in 21 subjects receiving treatment for homocystinuria. Of the 18 CBS-deficient individuals ranging in age from 8 to 58 years (mean, 30 years) in the study, 12 were pyridoxine-nonresponsive, with tHcy levels >75 μmol/L, yet none had ever suffered a vascular event. In 2 patients with CBS deficiency, it was possible to study the effect of B-complex treatment on tHcy and EC-SOD. There were highly significant reductions in both tHcy and EC-SOD during treatment for hyperhomocysteinemia. The same group had previously shown that low levels of EC-SOD were independently associated with risk and a history of myocardial infarction.²⁵,²⁶ Interestingly, Clarke et al²⁷ found that cytosolic red blood cell Cu,Zn-SOD was higher in obligate heterozygotes for CBS deficiency than in normal subjects, but the difference failed to achieve statistical significance owing to the small number of heterozygous subjects.

If oxidative stress due to hyperhomocysteinemia plays a role in vascular damage, then one might expect that there would be readily detectable oxidant stress markers in the sera from subjects with homocystinuria. However, older studies have been unable to document markers of oxidative stress in sera from subjects with homocystinuria.²⁸–³¹ Using a more sensitive indicator of oxidative stress, Voutilainen et al³² measured plasma F₂-isoprostanes as an in vivo indicator of lipid peroxidation in 100 male subjects. F₂-isoprostanes, which are derived from free radical–mediated lipid peroxidation reactions of arachidonic acid, increased linearly across tHcy quintiles. In a linear regression model, homocysteine had the strongest association with F₂-isoprostanes. Perhaps it is now time to reevaluate the status of oxidative stress markers in homocystinurics and hyperhomocysteinemic subjects with cardiovascular disease by using more sensitive techniques to assess lipid and protein oxidation.³³,³⁴

The hyperhomocysteinemic state, whether it be basal or transient due to methionine loading, is a reflection of metabolic events that occur within host cells. Intracellular disposal of homocysteine in the liver occurs by remethylation and transsulfuration pathways. However, the transsulfuration pathway has limited tissue distribution and is not expressed in human cardiovascular cells and tissues.³⁶ If homocysteine export fails to keep up with production, intracellular accumulation could become cytotoxic. One mechanism of cytotoxicity might involve the inactivation of the glutathione antioxidant defense system. In this scenario, the injury comes from within, not without, and may involve specific molecular targets. Nishio and Watanabe³⁷ have shown that treatment of rat aorta smooth muscle cells with L-homocysteine (0 to 500 μmol/L) decreased glutathione peroxidase activity but increased SOD activity in a dose-dependent manner. Homocysteine had no effect on catalase activity. Furthermore, homocysteine inactivated purified bovine liver glutathione peroxidase, again in a dose-dependent manner. Upchurch et al³⁸ found that 50 to 250 μmol/L L-cysteine inhibited glutathione peroxidase activity in cultured bovine aortic endothelial cells, but they did not obtain evidence for direct inactivation of the enzyme. They did observe that 5.0 mmol/L homocysteine (but not cysteine) decreased steady-state mRNA for glutathione peroxidase by 90%. Using cultured porcine aortic endothelial cells, Lang et al³⁹ showed that 0.03 to 1.0 mmol/L L-homocysteine but not L-cysteine or glutathione stimulated intracellular production of superoxide and induced higher levels of SOD activity. These studies suggest that “oxidative stress” may be generated within vascular cells, but not necessarily as a result of thiol oxidation.

Is homocysteine a mediator or marker of vascular disease? It may be several years before we know. The homocystinurics studied by Wilcken et al, many of whom have high tHcy levels, are an interesting yet perplexing group of subjects. Although this group has remained “event-free,” is there evidence for developing atherosclerosis, and is EC-SOD truly protective in these subjects? The study is provocative and a challenge to those concerned with the mechanism of homocysteine-induced vascular injury.

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Hyperhomocysteinemia and Oxidative Stress: Time for a Reality Check?
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