Hyperhomocysteinemia
A Million Ways to Lose Control
Frank M. Faraci

Elevated plasma levels of the amino acid homocysteine increase the risk for atherosclerosis, stroke, myocardial infarction, and possibly Alzheimer’s disease. In relation to vascular biology, many studies suggest that endothelial dysfunction contributes to the complex changes that occur within the vessel wall during hyperhomocysteinemia (HHCy). The underlying molecular mechanisms that are activated during HHCy have just begun to be understood.

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With regard to endothelial dysfunction, a major focus has been on mechanisms that impair NO-mediated signaling within the vessel wall (Figure). NO produced by the endothelial isoform of NO synthase (eNOS) is known to be the major endothelium-derived relaxing factor in blood vessels. Endothelium-dependent relaxation is impaired during HHCy in experimental animals and humans, in both large arteries and microvessels.1–8 Mechanisms that mediate this impairment are very complex, as they potentially involve every major component of eNOS signaling (Figure). An intriguing mechanism now receiving increased attention involves inhibiting the activity of eNOS by increased levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthases.9,10

Several lines of evidence suggest that increased oxidative stress and levels of reactive oxygen species play a key role in vascular changes elicited by HHCy. For example, pharmacological and genetic approaches that alter antioxidant levels provide strong support for the hypothesis that oxidative stress is a critical mechanism in HHCy-induced endothelial dysfunction.2,7,8,11–13 In addition to stimulating increased production of reactive oxygen species, HHCy can increase oxidative stress by inhibiting expression or function of key antioxidant enzymes such as extracellular superoxide dismutase (EC-SOD)(Figure).14,15

A major issue in relation to endothelial function relates to the bioactivity of NO which depends, in part, on its interaction with reactive oxygen species, particularly superoxide. Many studies have suggested that bioactivity of endothelium-derived NO is reduced during HHCy (Figure).2,7,8,11,13 In addition to inactivating NO and thus preventing normal NO-mediated signaling, the reaction of NO with superoxide produces peroxynitrite (ONOOH), a reactive nitrogen species and potent oxidant. Both superoxide and ONOOH are elevated within the vessel wall during HHCy.2,7,12,13 ONOOH can exert multiple detrimental effects, including tyrosine nitration of prostacyclin synthase and the major mitochondrial isoform of SOD (Mn-SOD), as well as activation of poly(ADP-ribose) polymerase (PARP). PARP activation is known to occur in nonvascular cells in response to homocysteine16 and activation of PARP may be an important mediator of vascular dysfunction in disease.17,18 ONOOH may also impair endothelial function by oxidizing tetrahydrobiopterin (Figure), leading to reduced activity of eNOS and/or eNOS “uncoupling,”19 a circumstance in which the normal flow of electrons within the enzyme is diverted so that eNOS produces superoxide rather than NO.

Although endothelium has received the most study, HHCy also affects other components and cell types within the vessel wall. HHCy alters expression of structural proteins and increases activity of matrix metalloproteinases in vessels.13 Recent work with genetically altered mice revealed that very modest increases in plasma homocysteine levels produce vascular hypertrophy in the microcirculation.20

With this background, the study in this issue of Arteriosclerosis, Thrombosis, and Vascular Biology by Ungvari et al21 used pharmacological and molecular approaches to better define mechanisms that promote oxidative stress in coronary arteries during HHCy. A rat model of diet-induced HHCy was studied, and as part of their approach, the investigators used a small signal transduction gene array to examine HHCy-induced changes in gene expression in the vasculature. There were several novel findings in the study. First, although previous work has demonstrated that superoxide is elevated in blood vessels in HHCy, the source or mechanism that accounts for the increase has not been defined. A major source of superoxide in vascular cells are NAD(P)H oxidas.22 HHCy produced an increase in Nox-1 [a gp91phox homologue and NAD(P)H oxidase subunit] expression and a parallel increase in NAD(P)H oxidase activity. Pharmacological inhibitors of NAD(P)H oxidase attenuated increases in superoxide in arteries from HHCy rats.

Second, HHCy is known to upregulate components of the inflammatory cascade, including activation of nuclear factor-κB (NF-κB), increased production of proinflammatory cytokines, and expression of adhesion molecules and monocyte chemoattractant protein.23–25 The study by Ungvari et al21 supports this concept as data obtained using real time RT-PCR, the gene array, and other approaches demonstrated that expression of the proinflammatory cytokine tumor ne-
crosis factor-α (TNFα) is increased in coronary arteries in response to HHCy.

TNFα may have many effects within vascular cells, including stimulation of expression of other inflammatory-related genes, inhibition of vasconstriction, and impairment of endothelial function. A recent report indicated that TNFα may contribute to endothelial dysfunction in patients with heart failure. The present findings related to HHCy support this concept, as they suggest that increased production of TNFα is responsible for a significant portion of the increase in NAD(P)H oxidase-derived superoxide that was seen in HHCy. This finding is consistent with earlier work demonstrating that TNFα increases activity of NAD(P)H oxidase in endothelium and vascular muscle in culture.

Third, previous studies have shown that inducible NO synthase (iNOS) can be expressed in vascular muscle in culture in response to homocysteine. The present finding that iNOS is also expressed in vessels in vivo is clearly of interest, although the functional importance of this expression has been difficult to predict. Depending on the cell type and system involved, iNOS has been reported to exert both protective and detrimental effects. Overexpression of iNOS using viral-mediated gene transfer produces impaired endothelial function. Impairment of endothelium-dependent relaxation in a model of diabetes and following lipopolysaccharide are absent in iNOS-deficient mice, suggesting that expression of iNOS plays a critical role in mechanisms that produce endothelial dysfunction in some disease states. A well-defined mechanism that accounts for this role of iNOS has not yet emerged. Although iNOS-derived NO may contribute importantly to formation of ONOO−, the role of superoxide in iNOS-mediated endothelial dysfunction has not been established. It is interesting that Ungvari et al provided pharmacological evidence that a portion of the increase in superoxide during HHCy was iNOS-derived suggesting that, in addition to NAD(P)H oxidase, iNOS may be an important source of superoxide.

In summary, the present study emphasizes the complexity regarding effects of HHCy within the vessel wall (Figure). Further emphasis on proinflammatory effects of homocysteine are evident as well as roles for both NAD(P)H oxidase and iNOS as sources of superoxide and a prominent role of TNFα in HHCy-induced vascular dysfunction.

Many questions remain however, in regard to effects of homocysteine in blood vessels. Although an important role for increased levels of reactive oxygen species and oxidative stress seems certain, the relative importance of different oxygen-derived species affecting different subcellular elements (proteins, transcription factors, lipids, etc) is poorly defined. Do increased levels of reactive oxygen species play a key role in mechanisms that produce HHCy-induced vascular hypertrophy? What is the functional importance of increased ONOO− in HHCy? Is ONOO− simply a passive marker of disease, or is it a direct mediator of vascular dysfunction via activation of PARP or other mechanisms? Although preliminary pharmacological data suggest that PARP may play a role, more definitive studies using PARP-deficient mice should be insightful.

Although eNOS- and NO-mediated signaling is clearly impaired, HHCy may impact other key signaling pathways as well. For example, initial evidence suggests that signaling that is mediated by peroxisome proliferator activated receptors (PPAR) is impaired with HHCy and that activation of PPAR may be beneficial. PPARs are transcription factors that regulate gene expression by binding to PPAR-response elements. Initial studies suggest that these transcription factors (via activation of their target genes) may exhibit multiple protective effects within the vessel wall.

Finally, although multiple processes are activated within the vessel wall and may contribute to vascular dysfunction and changes in wall structure during HHCy, it would be naive to assume that these different mechanisms each act independently of one another. For example, there is evidence that during diabetes, elevated levels of superoxide decrease activity of dimethylarginine dimethylaminohydrolase (DDAH)
resulting in decreased clearance and accumulation of ADMA. A similar mechanism is a reasonable candidate to account for elevated levels of ADMA in HHCy.

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References


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