Nitric oxide is a principal factor involved in the antiatherosclerotic properties of the endothelium.\textsuperscript{1–3} NO interferes in vitro with key events in the development of atherosclerosis, such as monocyte and leukocyte adhesion to the endothelium\textsuperscript{14–17} as well as platelet–vessel wall interaction.\textsuperscript{8–20} NO also decreases endothelial permeability and reduces vessel tone, thus decreasing flux of lipoproteins into the vessel wall.\textsuperscript{11,12} Finally, NO has been shown to inhibit vascular smooth muscle cell proliferation and migration in vitro as well as in vivo.\textsuperscript{15–16} In agreement with these findings, inhibition of the NO-producing enzyme NOS III caused accelerated atherosclerosis in experimental models.\textsuperscript{17} Major risk factors for atherosclerotic vascular disease, such as hypercholesterolemia, diabetes, hypertension, and smoking, have been associated with impaired NO activity.\textsuperscript{18–22}

In vivo, the activity of the L-arginine–NO pathway is a balance between the synthesis and breakdown of NO. At present, there are several reasons to believe that NO synthesis could indeed be impaired in hypercholesterolemia and atherosclerosis. In these conditions, there appears to be an uncoupling of the receptor–G, complex.\textsuperscript{23} The exact mechanism is not known, but a very intriguing hypothesis is that the altered lipid composition of the cell membrane may play a role in this phenomenon. Although there is some controversy regarding the effect of oxidized LDL on the transcription of the enzyme, there is evidence of reduced transcription and enhanced breakdown of NOS transcripts with increasing concentrations of oxidized LDL.\textsuperscript{24} Long-term stimulation with oxidized LDL may also lead to a decrease in the amount of the NOS protein through induction of cytokines.\textsuperscript{25} Finally, hypercholesterolemia is associated with increased circulating concentrations of ADMA, an endogenous inhibitor of NOS. This has been demonstrated in hypercholesterolemic rabbits and humans.\textsuperscript{26,27} This is particularly interesting because these observations suggest that administration of L-arginine may overcome a competitive inhibition of NOS.

In agreement with this theory, administration of L-arginine increases synthesis of NO by the vascular endothelium,\textsuperscript{28} improves NO-dependent vasodilation in conditions such as hypercholesterolemia and angina pectoris,\textsuperscript{18,29,30} and prevents development of atherosclerosis in LDL receptor knockout mice.\textsuperscript{31} These facts led to the concept that a reduction in NO synthesis is the primary process involved in endothelial dysfunction and that this reduction in NO synthesis is due to a reduced availability of the NOS substrate L-arginine. However, the intracellular concentration of L-arginine far exceeds the \( K_m \) value of NOS, making less likely the possibility that the extracellular L-arginine concentration is rate limiting.\textsuperscript{32} It is also uncertain to what extent increased circulating levels of ADMA affect intracellular L-arginine availability. Finally, a recent study by Giugliano et al\textsuperscript{33} demonstrates that the effect of L-arginine on vasodilation is mediated in part by stimulation of insulin secretion.

Alternatively, reduced NO activity could be caused by enhanced catabolism. The in vivo half-life of NO is determined mainly by its reaction with oxyhemoglobin and superoxide.\textsuperscript{34} The reaction of superoxide and NO occurs at a diffusion-limited rate, with the production of the powerful oxidant peroxynitrite (ONOO\textsuperscript{-}). This reaction is more than three times faster than catabolism of superoxide by superoxide dismutase.\textsuperscript{35} Under physiological conditions, NO is probably formed in the picomolar to nanomolar range.\textsuperscript{36} Because peroxynitrite is formed optimally from equimolar concentrations of NO and superoxide,\textsuperscript{36} it is not very likely for peroxynitrite to achieve high concentrations in normal physiology. This is important because low concentrations of peroxynitrite have been shown to behave very similarly to NO: they can cause vasorelaxation,\textsuperscript{37–39} decrease platelet aggregation,\textsuperscript{38,40} reduce leukocyte adhesion to the vessel wall,\textsuperscript{41} exert cytoprotective effects,\textsuperscript{42} and in fact may act as an NO donor.\textsuperscript{43} By contrast, higher concentrations of peroxynitrite may be very toxic. It can form the cytotoxic peroxynitrous acid,\textsuperscript{44} cause hydroxyl radical toxicity,\textsuperscript{45} and cause protein fragmentation by nitrination of amino acids.\textsuperscript{45} It can be postulated that such deleterious concentrations of peroxynitrite can be achieved in atherosclerotic lesions, in which superoxide generation is increased by endothelial oxidases such as xanthine oxidase\textsuperscript{46,47} as well as oxidase systems in infiltrating leukocytes (see Fig 1), while at the same time NO production in atherosclerotic lesions may also be increased by induction of NOS II by cytokines.\textsuperscript{48} Moreover, hypercholesterolemia, as a risk factor for atherosclerosis, has been shown to impair the glutathione detoxification mechanism against peroxynitrite.\textsuperscript{49} In agreement, nitrotyrosine immunostaining, which has been advanced as a marker of peroxynitrite-mediated protein modification,\textsuperscript{34} is increased in human atherosclerotic plaques. Moreover, nitrosylation of LDL cholesterol isolated from atherosclerotic plaques is also largely increased,\textsuperscript{50} suggesting...
that enhanced peroxynitrite formation occurs in atherosclerosis and could contribute to lipid peroxidation in atherosclerosis.\textsuperscript{24,51} Taken together, these data indicate that catabolism of NO by its reaction with superoxide could be an important phenomenon in hyperlipidemia and atherosclerosis.

Very intriguing are observations that NOS III itself can be an important source of endothelial superoxide production in hypercholesterolemia. Pritchard et al\textsuperscript{53} found that endothelial cells that were incubated with LDL released superoxide, which could be largely inhibited by the NOS inhibitor L-NAME. In fact, superoxide production by the endothelium fell below control levels during administration of L-NAME, suggesting that there is continuous superoxide production by NOS III. In other words, NOS III is both an NO- as well as a superoxide-producing enzyme. In view of the fast reaction of NO with superoxide, one could even postulate that NOS III is to some extent a peroxynitrite-producing enzyme. To understand this double action of NOS III, one has to take a closer look at its biochemistry.

The Two Faces of NOS

NOS III consists of a flavin-containing reductase domain and a heme-containing oxidase domain\textsuperscript{74} (Fig 2). NADPH reduces molecular oxygen into the substrate L-arginine. NOS III is present as a homodimer. The NOS III also undergoes posttranslational acylation (myristoylation and palmitoylation), which appears to be essential for its activity, by anchoring the enzyme to the cell membrane.\textsuperscript{55} The NOS III probably binds to distinct domains of the plasma membrane, called caveolae, which may serve as sites for the sequestration of receptor-coupled signaling proteins and which are tethered to the cytoskeleton. Recently, it has been shown that interaction of NOS III with caveolin-1 has an inhibitory effect on enzyme activity, while this interaction may be reversed by calcium-calmodulin activation.\textsuperscript{55a} The NOS III itself (bottom) has binding sites for BH\textsubscript{4}, L-arginine, and heme. Electrons, donated by NADPH, are transported toward the oxidase domain. Heme may reduce molecular oxygen, leading to the formation of superoxide. The electrons are donated to the aminoguanidine group of L-arginine, leading to the formation of NO and L-citrulline. This reaction is dependent on the presence of BH\textsubscript{4}.

\textbf{Figure 1.} Proposed scheme of catabolism of NO by superoxide (O\textsubscript{2}•\textsuperscript{-}). Under physiological conditions, endothelial NOS III produces NO as well as superoxide (see text). NO may react rapidly with superoxide generated by mitochondria and membrane-bound oxidase systems such as xanthine oxidase (XO) to form peroxynitrite (ONOO•), which at low concentrations has very similar actions to NO. In atherosclerosis (bottom), the NOS III produces less NO and more superoxide, while at the same time superoxide generation by XO and possibly NAPDH oxidases is also increased. In addition, there is increased superoxide formation by leukocytes through, for example, myeloperoxidases (MPO) and lipoygenase (lipox). If this situation is accompanied by induction of NOS II, concomitant increments in NO and superoxide release by NOS II may lead to the formation of high concentrations of peroxynitrite, a condition that has been associated with cellular toxicity.
Figure 3. Effects of BH4 on serotonin (5-HT)-induced NO-mediated vasodilation in controls but significantly enhanced 5-HT-mediated vasodilation in young patients with familial hypercholesterolemia (FH) without macrovascular disease. There was no effect of BH4 on sodium nitroprusside (SNP)-induced endothelium-independent vasodilation (reprinted with permission).
detoxification mechanism against peroxynitrite and renders the vascular tissue more susceptible to oxidative injury. *Circ. Res.* 1997;80:894–901.


52. Deleted in proof.


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Atherosclerosis and the Two Faces of Endothelial Nitric Oxide Synthase
Robert M. F. Wever, Thomas F. Lüscher, Francesco Cosentino and Ton J. Rabelink

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