Nitric oxide is a soluble gas continuously synthesized by the endothelium. This substance has a wide range of biological properties that maintain vascular homeostasis, including modulation of vascular dilator tone, regulation of local cell growth, and protection of the vessel from injurious consequences of platelets and cells circulating in blood. A growing list of conditions, including those commonly associated as risk factors for atherosclerosis such as hypertension and hypercholesterolemia, are associated with diminished release of nitric oxide into the arterial wall either because of impaired synthesis or excessive oxidative degradation. Diminished nitric oxide bioactivity may cause constriction of coronary arteries during exercise or during mental stress and contribute to provocation of myocardial ischemia in patients with coronary artery disease. Additionally, diminished nitric oxide bioactivity may facilitate vascular inflammation that could lead to oxidation of lipoproteins and foam cell formation, the precursor of the atherosclerotic plaque. Numerous therapies have been investigated to assess the possibility of reversing endothelial dysfunction by enhancing the release of nitric oxide from the endothelium, either through stimulation of nitric oxide synthesis or protection of nitric oxide from oxidative inactivation and conversion to toxic molecules such as peroxynitrite. Accordingly, causal relationships between improved endothelial function and reduction in myocardial ischemia and acute coronary events can now be investigated.

Far from being only an anatomic barrier to prevent the extravasation of circulating blood into the vessel wall, the endothelium is a metabolically active organ system that maintains vascular homeostasis by (a) modulating vascular tone, (b) regulating solute transport into cell components of the vessel wall, local cellular growth, and extra-cellular matrix deposition, (c) protecting the vessel from the potentially injurious consequences of substances and cells circulating in blood, and (d) regulating the hemostatic, inflammatory, and reparative responses to local injury. However, a growing list of conditions, including hypercholesterolemia, systemic hypertension, smoking, diabetes, congestive heart failure, pulmonary hypertension, estrogen deficiency, hyperhomocysteinemia, and the aging process itself, have been associated with impaired functions of the endothelium. As a result, the vessel wall in these conditions may promote inflammation, oxidation of lipoproteins, smooth muscle proliferation, extracellular matrix deposition or lysis, accumulation of lipid-rich material, platelet activation, and thrombus formation. All of these consequences of endothelial dysfunction may contribute to development and clinical expression of atherosclerosis.

The Central Regulatory Role of Nitric Oxide
Nitric oxide is a soluble gas with a half-life of \(~6–30\) s, continuously synthesized from the amino acid \(l\)-arginine in endothelial cells by the constitutive calcium-calmodulin-dependent enzyme nitric oxide synthase (1). This heme-containing oxygenase catalyzes a five-electron oxidation from one of the basic guanido nitrogen atoms of \(l\)-arginine in the presence of multiple cofactors and oxygen. In their seminal experiment, Furchgott and Zawadzki (2) found that strips of rabbit aorta with intact endothelium relaxed in response to acetylcholine but constricted in response to the same agonist when the endothelium had been rubbed off. The substance responsible for the acetylcholine-stimulated relaxation was initially called endothelium-derived relaxant factor, and subsequently found to include nitric oxide (3, 4). It is now known that a variety of agonists (e.g., acetylcholine, histamine, thrombin, serotonin, ADP, bradykinin, norepinephrine, substance \(P\), and isoproterenol) can increase the synthesis and release of nitric oxide from the endothelium, although many of these same agonists (e.g., acetylcholine, serotonin, norepinephrine, and histamine) constrict vascular smooth muscle in the absence of endothelium. Vasoactive substances produced within the
endothelium, such as bradykinin, may also stimulate nitric oxide release by autocrine and paracrine effects on endothelial B2 kinin receptors (5). However, the principal physiologic stimulus for nitric oxide synthesis and release from the endothelium is likely the shear stress of blood flowing over the surface of the vessel by a nonreceptor-dependent mechanism (6, 7). Nitric oxide, released from the endothelium as a gas or attached to other molecules, stimulates soluble guanylyl cyclase, producing increased concentrations of cyclic GMP. Depending on the direction of nitric oxide release and the site of cyclic GMP activation, differing biological effects can be observed. For example, increased cyclic GMP in vascular smooth muscle cells underlying the endothelium activates GMP-dependent kinases that decrease intracellular calcium, producing relaxation (8), whereas increased cyclic GMP in platelets by action of nitric oxide released into the blood vessel lumen decreases platelet activation and adhesion to the surface of the endothelium (9). Nitric oxide also regulates the cellular environment within the vessel wall by inhibiting the activity of growth factors released from cells within the vessel wall and from platelets on the endothelial surface (10). Nitric oxide has antiinflammatory properties by inhibiting the synthesis and expression of cytokines and cell adhesion molecules that attract inflammatory cells to the endothelial surface and facilitate their entrance into the vessel wall (11, 12). This effect of nitric oxide may be mediated by inhibition of the activation of an important nuclear transcription factor (nuclear factor kB) that binds to the promoter regions of genes that code for proinflammatory proteins (12). Nitric oxide also governs basal systemic, coronary, and pulmonary vascular tone by increased cyclic GMP in smooth muscle, by inhibition of a potent constrictor peptide, endothelin-1 (13), and by inhibition of the release of norepinephrine from sympathetic nerve terminals (14).

Thus, nitric oxide plays a pivotal role in regulating vessel wall homeostasis. Although the endothelium-dependent processes to be discussed involve a multitude of metabolic and gene transcriptional pathways, nitric oxide either directly or indirectly plays an important role in their regulation.

Nitrergic Oxidative and Vasomotor Tone in Healthy Subjects
The importance of nitric oxide as a regulator of coronary vasomotor tone can be demonstrated experimentally by inhibiting its synthesis. Thus, N\textsuperscript{G}-monomethyl-\textit{l}-arginine (L-NMMA), which competes with \textit{l}-arginine as the substrate for nitric oxide synthesis by the enzyme nitric oxide synthase but cannot be oxidized to form nitric oxide (15), increases basal coronary vascular resistance and blunts the vasodilator response to the endothelium-dependent vasodilator agonists acetylcholine and bradykinin in isolated perfused hearts (16, 17). These responses to nitric oxide inhibition are reversible by the addition of \textit{l}-arginine. L-NMMA administered systemically to the awake dog at doses that increase systemic blood pressure by blocking nitric oxide production in the systemic circulation also increases coronary vascular resistance (18), suggesting that nitric oxide release may be of physiological importance in the regulation of basal systemic and coronary tone, especially at the level of the arterioles (resistance vessels) in these vascular distributions.

Investigators at the National Institutes of Health have conducted studies to determine the contribution of nitric oxide to basal vascular tone in humans. Panza et al. (19) measured forearm blood flow by strain gauge plethysmography, before and after inhibition of nitric oxide synthesis in the forearm with intraarterial infusion of L-NMMA. L-NMMA reduced forearm blood flow, a vasconstrictor effect suggesting substantial contribution of nitric oxide to the basal dilator tone of forearm resistance vessels. L-NMMA also blunted the vasodilator response to the intraarterial infusion of acetylcholine, suggesting that this agonist stimulates the release of nitric oxide from the endothelium (Fig. 1). Quyyumi et al. (20) found that infusion of L-NMMA into coronary arteries of patients with normal coronary angiograms and no risk factors for coronary atherosclerosis (who were considered to be healthy subjects) reduced the epicardial coronary diameter by 14% and coronary blood flow by 19% (calculated from intracoronary Doppler flow velocity measurements and quantitative angiography; Fig. 2). Because this mild degree of epicardial coronary artery constriction should not affect coronary blood flow, this suggests that in the normal coronary circulation, nitric oxide contributes to both basal epicardial and arteriolar dilator tone. In these healthy subjects, the vasodilator response to intracoronary infusion of acetylcholine at both the epicardial and the microvascular levels of coronary circulation was significantly attenuated by L-NMMA (Fig. 3), suggesting that, just as was found in the forearm circulation, acetylcholine stimulates the release of nitric oxide from the coronary endothelium.

Although the systemic and coronary vasodilator responses to acetylcholine were substantially attenuated with L-NMMA in these studies, the responses were not entirely abolished, suggesting that relaxant factors other than nitric oxide may also be released from the endothelium in response to acetylcholine. Among the endothelium-derived non-nitric oxide substances that cause smooth muscle relaxation is prostacyclin, which activates adenylyl cyclase, increasing smooth muscle cyclic AMP (21). Another vasodilator action of the endothelium is mediated by the release of substances— including nitric oxide, prostacyclin, and eicosatricine acid—that hyperpolarize smooth muscle by activating calcium-dependent potassium channels and are collectively referred to as endothelium-derived hyperpolarizing factors (22). These substances are released from the endothelium by many of the same agonists (e.g., acetylcholine and bradykinin) that stimulate nitric oxide synthesis after receptor-activated increases in endothelial cytosolic calcium concentration.
Nitric Oxide During Stress
During physical and mental stress, increases in coronary blood flow because of sympathetically mediated increases in cardiac output also augment shear stress across the endothelium, producing coronary and systemic arterial dilation (23–27). Vasodilation during stress may also be mediated by epinephrine and norepinephrine activation of adrenoceptors on the endothelium, with enhanced synthesis and release of nitric oxide (27–28). However, the contribution of shear stress to coronary arteriolar dilator responsiveness, and thus to the regulation of coronary blood flow appropriate to the metabolic demands of stress, is unclear. Unlike epicardial arteries, the microcirculation of the heart is under control of the surrounding metabolic environment. During stress, increased release of a variety of substances by the myocardium (such as adenosine) and activation of ATP-sensitive potassium channels produce coronary arteriolar smooth muscle relaxation, and thus dilation, independent of the endothelium. In this regard, inhibition of nitric oxide synthesis does not impair the coronary flow response to rapid atrial pacing or to exercise in dogs (29–30). This redundancy of vasodilator mechanisms in the coronary circulation at the arteriolar sites of blood flow regulation is not surprising, given the survival value of adequate coronary vasodilator responses and thus appropriate coronary blood flow delivery to the myocardium during physiological stresses.

Nitric Oxide and Hypertension
Hypertension in most patients is associated with sustained increases in systemic arteriolar tone compared with normotensive subjects. Panza et al. (19) found that L-NMMA infused into the brachial artery had less of an effect on basal forearm flow compared with normotensive subjects, suggesting that basal release of nitric oxide is deficient in hypertension (Fig. 4). They had found previously that the increase in forearm flow in response to acetylcholine was blunted in hypertensive subjects compared with the acetylcholine responses in normotensive subjects (31). L-NMMA had minimal effect on the forearm flow response to acetylcholine, suggestive of defective release of nitric oxide on endothelial stimulation (19). However, the vasodilator response to nitroprusside, which acts directly on smooth muscle independently of the endothelium by the direct release of nitric oxide from this compound, was not different from the response of normotensive subjects, indicating preserved smooth muscle responsiveness to nitric oxide in hypertension. Deficient vasodilator responses to other receptor-activated endothelium-dependent agonists—substance P and bradykinin—have also been demonstrated in hypertensive subjects, suggesting that a selective defect in G-protein-dependent intracellular signal transduction pathways is not the mechanism of endothelial dysfunction in these patients (32, 33). Recent work by this group suggests that defective function of these agonists of G-protein-dependent pathways that are activated by phosphoinositol-specific phospholipase C may be of particular pathogenetic importance in hypertension: Isoproterenol activation of a G-protein-dependent pathway that activates adenyl cyclase is not impaired in hypertensive subjects (34).
Nitric Oxide and Hypercholesterolemia

In hypercholesterolemic subjects, L-NMMA has similar effect on basal forearm flow compared with normocholesterolemic subjects. However, the effect of L-NMMA on the forearm flow response to acetylcholine was reduced compared with normocholesterolemic subjects, suggesting preserved release of nitric oxide in the basal state but reduced nitric oxide activity during endothelial stimulation (35). However, unlike the impaired vasodilator response to bradykinin noted in hypertensive patients (33), the response to bradykinin in hypercholesterolemic subjects was found to be similar to the response of healthy subjects, suggestive of selective impairment of a G-protein-dependent pertussis toxin-sensitive signal transduction pathway in hypercholesterolemia (36).

Nitric Oxide and Atherosclerosis

Several groups have shown that epicardial coronary arteries in patients with coronary artery disease constrict both at sites of angiographically obstructive atherosclerotic disease and at sites of plaquing in response to acetylcholine (37–41). These same doses of acetylcholine cause vasodilation in coronary arteries of patients without evidence of coronary artery disease. In contrast to the different responses of these two patient groups to acetylcholine, the responses to nitroglycerin (a nitric oxide donor) are similar, indicating intact smooth muscle responsiveness to nitric oxide, at least in mildly atherosclerotic arteries. Even risk factors for atherosclerosis such as hypercholesterolemia, male sex, age, smoking, diabetes mellitus, and a family history of coronary artery disease have been associated with constrictor responses of the epicardial coronary arteries to acetylcholine in patients with normal-appearing coronary angiograms (20, 41–44). These observations suggest that endothelial dysfunction of epicardial coronary arteries precedes development of atherosclerotic disease that is either angiographically apparent or of sufficient obstructive severity to cause myocardial ischemia and angina pectoris.

To investigate the mechanism of coronary endothelial dysfunction in early atherosclerosis, Quyyumi and co-workers (20, 44) studied patients with normal-appearing...
coronary angiograms but who had multiple risk factors for atherosclerosis (hypertension, hypercholesterolemia, smoking, diabetes, and aging), comparing their coronary vascular responses to intracoronary infusion of acetylcholine with the responses of patients with angiographically apparent coronary atherosclerosis and with patients with normal-appearing coronary angiograms who were free of risk factors and who served as healthy controls. In contrast to constriction of epicardial coronary arteries and decreased coronary flow demonstrated in healthy subjects after inhibition of nitric oxide synthesis with L-NMMA, the constrictor effects after intracoronary infusion of L-NMMA were reduced in patients with risk factors for atherosclerosis (Fig. 2) and in patients with coronary atherosclerosis (Fig. 3). Similarly, epicardial coronary arteries dilated with acetylcholine in healthy subjects, but constricted in patients with atherosclerosis and patients with risk factors for atherosclerosis. L-NMMA inhibited the coronary arteriolar dilator response to acetylcholine in all groups, but the magnitude of inhibition was significantly greater in healthy subjects compared with patients with atherosclerosis and patients with risk factors for atherosclerosis. Similarly, L-NMMA-induced inhibition of epicardial diameter change with acetylcholine was greater in healthy subjects than the two patient groups. These observations indicate that the reduced dilator response to acetylcholine in patients with atherosclerosis and in patients with risk factors for atherosclerosis is because of impaired acetylcholine-induced nitric oxide release from the endothelium. Coronary epicardial and arteriolar dilation were similar in response to the endothelium-independent vasodilators nitroprusside and adenosine in healthy subjects and in patients with atherosclerosis or risk factors for atherosclerosis, except for a mild reduction in atherosclerotic vessel dilation in response to sodium nitroprusside in the most severely
stenotic arteries. L-NMMA did not inhibit vasodilation in response to sodium nitroprusside or adenosine, indicating that the abnormal reactivity observed with acetylcholine in patients with risk factors for atherosclerosis was specific for the endothelium.

Mechanisms and Consequences of Endothelial Dysfunction
The acute and chronic manifestations of atherosclerosis are increasingly being considered to be a consequence of a chronic inflammatory process, possibly initiated and perpetuated in part by LDL that is trapped and oxidized within the vessel wall (45–48). Through activation of the transcription factor nuclear factor \(\kappa_B\), oxidized LDL induces the synthesis and expression of adhesion molecules on the endothelial cell surface that tether circulating inflammatory cells to the endothelium and facilitate their entry into the vessel wall (49). These inflammatory cell adhesion molecules, once expressed on the endothelial cell surface, may be shed from the cell surface. In this regard, serum concentrations of \(\alpha\)-selectin, vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 have been reported to be higher in patients with coronary artery disease than in healthy controls (50–52). Once in the vessel wall, inflammatory cells may release highly reactive oxygen-derived free radical molecules (such as superoxide anion) that oxidize lipoproteins. Tissue macrophages transformed from circulating monocytes and smooth muscle cells then take up oxidized LDL, becoming foam cells, the earliest histologic feature of atherosclerosis.

In addition to inflammatory cells, endothelial cells in hypercholesterolemic animal models of atherosclerosis may also produce increased quantities of highly reactive molecules such as superoxide anion (53). Recent work has shown that constitutive nitric oxide synthase in endothelial cells in culture can generate large quantities of superoxide anions after addition of LDL to culture media (54). Vascular smooth muscle cells in rats made hypertensive with angiotensin II also generate superoxide anions because of activation of cell membrane-associated NADH/NADPH oxidase (55). Superoxide anions and free radical molecules may oxidize nitric oxide to metabolites that do not activate guanylyl cyclase and that are potentially harmful to the endothelium (e.g., peroxynitrite). What is unclear is whether the actual synthesis of nitric oxide by the dysfunctional endothelium in hypercholesterolemia is increased or decreased. In support of the possibility of increased nitric oxide formation, the release of nitrogen oxides is increased from atherosclerotic rabbit aorta compared with control tissue (56). LDL added to endothelial cells in culture stimulates the release of nitrogen oxides (especially peroxynitrite) (54). Oxidized LDL has been shown to stimulate the transcription and synthesis of nitric oxide synthase (57). And finally, increased expression of the inducible form of nitric oxide synthase, capable of synthesizing even larger quantities of nitric oxide than the constitutive form of this enzyme, has been detected in human atherosclerotic plaques (58). Thus, vascular cells in hypercholesterolemia and atherosclerosis may synthesize greater quantities of nitric oxide than nondiseased cells, but with rapid oxidative inactivation or conversion to toxic nitrogen oxides because of excess accumulation of superoxide anions and free radical molecules.

On the other hand, oxidized LDL may impair signal transduction activation of nitric oxide synthase, thus diminishing the synthesis of nitric oxide (59, 60). In addi-

![Fig. 4. Patients with hypertension have reduced forearm blood flow responses to acetylcholine compared with normotensive controls. Data given as mean ± SE. Reprinted from Panza et al. (31) with permission from the Massachusetts Medical Society.](image-url)
tion, competitive inhibitors of nitric oxide synthase may be synthesized in the endothelium under certain conditions. In this regard, asymmetric dimethylarginine, which competes with l-arginine as the substrate for nitric oxide synthesis, thus inhibiting enzyme activity, has been detected in hypercholesterolemic humans (61). Increased concentrations of lipoprotein(a) have also been associated with impaired coronary endothelial function (62, 63), possibly through the inhibitory effects of oxidized components of this lipoprotein on nitric oxide synthesis or by oxidation and inactivation of nitric oxide (64). Nitric oxide release may also be reduced because of oxidation by glycosylation products produced in large quantities within the vasculature of diabetics and cigarette smokers (65).

In patients with hypercholesterolemia and in patients with coronary atherosclerosis, coronary and systemic arteries may constrict during exercise (23, 24, 66) or with mental stress (25–27), probably because of loss of dilator regulation by the coronary endothelium as a consequence of diminished release of nitric oxide to the vascular smooth muscle, whether by decreased synthesis or excess degradation, and enhanced vascular sensitivity to constrictor stimuli such as norepinephrine (26, 27). Reduced nitric oxide could also stimulate the synthesis and release of endothelin, producing enhanced vasoconstrictor tone; promote the release and activity of growth factors, producing smooth muscle hyperplasia and migration into the intima; and enhance the synthesis and release of proinflammatory cytokines. Additionally, reduced nitric oxide could promote platelet attachment and release of growth factors in the vessel wall. All of these consequences of endothelial dysfunction and reduced nitric oxide bioactivity may be important in the initiation, progression, and clinical expression of atherosclerosis.

The Coronary Endothelium after Acute and Chronic Ischemia

Reperfusion after prolonged ischemia in animals is associated with limitation in myocardial perfusion, probably caused, in part, by injury to the endothelium of the coronary microcirculation (68, 69). The microvascular endothelium may be more vulnerable to the effects of ischemia and reperfusion than the epicardial coronary arterial endothelium (70, 71). Damaged endothelium may limit vasodilation or promote vasoconstriction in response to circulating and platelet-derived substances, permit adhesion and ingress of inflammatory cells into the vessel wall, promote platelet activation, promote the release of oxygen free radical molecules, activate local procoagulant mechanisms, and contribute to the formation of microthrombi (72). All of these deleterious effects could exacerbate ischemic myocardial injury and necrosis and contribute to or prolong impaired myocardial systolic function (stunning), thus compromising the success of thrombolytic or mechanical reperfusion in humans. In this regard, nitric oxide reduced the extent of necrosis after 1 h of ischemia followed by 4.5 h of reperfusion in the open-chest dog, with reduction in neutrophil adherence to the coronary endothelium (73).

Reversibility of Coronary Endothelial Dysfunction

Numerous therapies have been examined to assess the possibility of reversing endothelial dysfunction by enhancing the release of nitric oxide from the endothelium either through stimulation of nitric oxide synthesis or protection of nitric oxide from oxidative inactivation and conversion to toxic molecules. For example, several groups have reported improvement in acetylcholine-stimulated coronary blood flow and prevention of acetylcholine-induced epicardial coronary artery constriction after intracoronary infusion of l-arginine, the substrate for nitric oxide synthesis, in patients with coronary artery disease and in patients with normal-appearing epicardial coronary arteries and who have risk factors for atherosclerosis (Fig. 5) (74–76). Intracoronary infusion of 17β-estradiol, achieving physiologic concentrations in the coronary sinus of estrogen-deficient postmenopausal women, improved coronary epicardial and arteriolar endothelium-dependent vasodilator responses to acetylcholine in estrogen-deficient postmenopausal women without altering endothelium-independent vasodilator responses (77). Because these effects of estrogen were blocked by L-NMMA, estrogen-mediated vasodilation appears to be because of enhanced nitric oxide release from the endothelium (Fig. 6) (78). Several groups have shown improvement in coronary and systemic vasodilator responses to acetylcholine after lipid-lowering and antioxidant (e.g., vitamin C) therapies (79–86). In the study by Anderson et al. (87), the magnitude of improvement in the epicardial coronary response to acetylcholine after therapy (i.e., prevention of coronary artery constriction observed before therapy) correlated strongly with protection of patients’ LDL from oxidation. Inhibition of xanthine oxidase, a potent generator of superoxide anion free radical molecules, with oxypurinol improved forearm endothelial responsiveness to acetylcholine in hypercholesterolemic patients (88). Angiotensin-converting enzyme therapy with quinapril prevented acetylcholine-induced constriction of epicardial coronary arteries of patients with coronary artery disease (89). Other therapies under investigation include tetrahydrobiopterin (a cofactor for nitric oxide synthase), non-vitamin antioxidants, and exercise.

Conclusion

An understanding of the homeostatic function of the vascular endothelium is important for the modern cardiologist. The role of nitric oxide in mediating many of the regulatory properties of the endothelium is now recognized, as is a growing understanding of how conditions and diseases considered to be “risk factors” for atherosclerosis cause endothelial dysfunction with loss of nitric oxide bioactivity. The potential consequences of endothelial dysfunction are numerous, including coronary constriction or inadequate dilation during physical or mental
Fig. 5. L-Arginine, the substrate for nitric oxide synthesis, enhances the coronary flow response to acetylcholine in patients with coronary artery disease. Data given as mean ± SE. Reprinted from Quyyumi et al. (76) with permission from the American College of Cardiology.

Fig. 6. 17β-Estradiol prevents the epicardial coronary constrictor response to acetylcholine and enhances the coronary blood flow response to acetylcholine in postmenopausal women. This effect of estradiol is blocked by L-NMMA, suggesting that the effect of estradiol on the coronary vasculature is mediated through enhanced bioactivity of nitric oxide. Data given as mean ± SE. Reprinted from Guetta et al. (78) with permission from the American Heart Association.
stress, producing myocardial ischemia; plaque rupture and thrombosis, causing unstable angina or myocardial infarction; and reperfusion injury after thrombolysis. The clinical implications of reversing endothelial dysfunction remain to be demonstrated in humans, but several studies suggest that lipid-lowering therapies reduce myocardial ischemia in patients with coronary artery disease. (90–92) Because many therapies appear capable of improving endothelial dysfunction, causal relationships between improved endothelial function and reduction in myocardial ischemia and acute coronary events can now be investigated.

References


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