Review Paper

Aspects of Nitric Oxide in Health and Disease: A Focus on Hypertension and Cardiovascular Disease

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Nitric oxide (nitrogen monoxide) (NO) plays an important role in a wide range of physiologic processes. A major mediator of endothelial function, NO regulates vasodilatory and antithrombotic actions in the vasculature and plays a role in reproductive functions, bronchodilation, bone formation, memory, insulin sensitivity, and gastrointestinal relaxation. NO is formed from NO synthase. Impaired NO bioactivity is strongly associated with endothelial dysfunction and cardiovascular disease, but is also implicated in a broad range of other disorders, including pulmonary hypertension, insulin resistance, erectile dysfunction, and preeclampsia. Numerous therapies designed to target NO are being investigated and developed, including NO donors and stimulants. The recent African-American Heart Failure Trial (A-HeFT) showed that the NO donor isosorbide dinitrate, combined with the vasodilator hydralazine, significantly reduced morbidity and mortality in black patients with moderate-to-severe heart failure. Antihypertensive drugs, including angiotensin-converting enzyme inhibitors, calcium channel blockers, and third-generation β-blockers, are NO stimulants that have demonstrated significant improvement of endothelial function and NO bioactivity. Other cardiovascular therapies that may improve NO bioactivity include statins, L-arginine, and nonpharmacologic approaches such as exercise and dietary changes. (J Clin Hypertens. 2006;8(12 suppl 4):2–16) ©2006 Le Jacq

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Nitric oxide (nitrogen monoxide) (NO) is a gas that is widely distributed throughout the human body and plays strikingly diverse roles in a broad spectrum of physiologic and pathophysiologic processes.[1,2] NO is a free radical and highly reactive molecule and is now recognized as an important signaling molecule or neurotransmitter, active in practically every major organ system, not only in mammals, but also in plant, fish, and insect species.[1,2]

In humans, NO is an important mediator of functions related to the cardiovascular (CV) system. In the endothelium, NO has emerged as a major factor in mediating vascular tone and exerting an antithrombotic action. Protection of target organs (eg, heart, brain, and kidneys) is also a function of NO.[1–3] Other functions of NO related to CV function and related metabolic activity include peripheral nervous system functions in the respiratory tract, sexual function, insulin release, and immune responses in inflammation.[1,2,4–8] NO is also believed to potentiate the protective benefits of high-density lipoprotein cholesterol in preventing atherosclerosis[7] and exercise in reducing the risks of CV disease.[8]

Impairment of NO bioactivity is strongly associated with endothelial dysfunction, hypertension, atherosclerosis, and CV disease,[3,9,10] and has been linked to a wide range of other disorders, including asthma and pulmonary hypertension,[5,11] erectile dysfunction,[12] preeclampsia,[13] and insulin resistance.[14]

NO often plays a paradoxical role as both a protective and pathogenic factor in multiple diseases, depending on the environment and pathway of NO production and expression.[1,2,5,6,15] The multiple functions of NO and the potential methods for therapeutically maintaining or restoring its optimal production and function have been investigated over the past 25 years.[16,17] This article reviews some of the major developments in this rapidly advancing field, chiefly as it pertains to the CV system in general and hypertension in particular.

HISTORICAL BACKGROUND

The CV effects of NO were first highlighted in experiments to determine the mechanism for the vasodilating effects of acetylcholine (ACh).[1,2] In animal studies, ACh was observed to cause dilation of arteries supplying blood to skeletal muscle in vivo; researchers hypothesized that this effect was due to diffusion of ACh to the vascular smooth muscle cells.[1] Furchgott and Zawadzki,[18] however, found in a landmark experiment that accidental rubbing of the intimal surface of an isolated rabbit aorta preparation abolished the vasodilatory effect of ACh.[18] The investigators hypothesized that rubbing of the intima caused loss of endothelial cells, which was supported by their observation that removal of the adventitia did not affect the vasodilatory action of ACh.[18] This vasodilatory action thus appeared to be dependent on the production by endothelial cells of an additional substance that diffused into the arterial smooth muscle cells and induced relaxation; this hypothetical substance was called endothelium-derived relaxation factor, and was distinct from endothelium-derived hyperpolarizing factor.

In seeking to identify endothelium-derived relaxation factor, Furchgott and Zawadzki eliminated several substances from their search then associated with endothelial function, including bradykinin, prostacyclin, 3',5'-cyclic guanosine monophosphate, and cyclic adenosine monophosphate.[1,18] Subsequent studies identified endothelium-derived relaxation factor as NO.[19,20]

SYNTHESIS AND MECHANISM OF ACTION

The production of NO is catalyzed by NO synthases (NOS), which convert the amino acid L-arginine to L-citrulline and NO.[2,21] NOS are members of a family of cytochrome P450–like reductases linked to a nicotinamide adenine dinucleotide phosphate (reduced) (NAD[P]H) oxidase enzyme. At least 3 isoforms, specific to different organ systems, exist. Endothelial NOS (eNOS), a constitutive isoform, is primarily expressed in the endothelium and airway epithelium. Neuronal NOS (nNOS), a second constitutive isoform, is present in the central and peripheral nervous systems, as well as skeletal muscle. Both eNOS and nNOS are calcium–calmodulin-dependent.[2,22] The third known NOS isoform is inducible NOS (iNOS), which is primarily produced by the action of endotoxin inflammatory cytokines such as interleukin 1 or tumor necrosis factor α in macrophages and other cell types.[2,22] All 3 of the isoforms, however, have been found in a variety of tissues, and the constitutive isoforms may also be inducible.[23]

Pulsatile blood flow and shear stress are the primary physiologic stimuli of NO production by the endothelium.[9] These forces open calcium channels on endothelial cells, thus promoting the calcium-dependent activation of eNOS, which, in turn, induces the release of NO.[13] Once synthesized, NO diffuses into the underlying vascular smooth muscle, where it activates soluble guanylate cyclase, causing an increase in 3',5'-cyclic guanosine monophosphate and smooth muscle relaxation.[2,9] The small size, lipophilic nature, and chemical lability of NO allow it to diffuse readily across cell membranes without need for channels or receptors.[13]

It had previously been thought that after diffusing into the blood stream, NO was bound to the hemoglobin in red blood cells.[24] Hemoglobin, however, reacts with NO in such a way that facilitates the redelivery of NO to the vessel wall as a biologically active *S*-nitrosothiol molecule.[24,25] Furthermore, this mechanism of preserved NO bioactivity is upregulated in oxygen-deprived tissues that are most in need of NO-induced vasodilation and increased blood flow.[25]

The mechanisms of stimulation of NO synthesis by nNOS are not well understood.[26] Study data suggest, however, that NO production by nNOS in the brain is increased in response to stress and hypoxia, where it is regulated by interacting proteins, and that 60% of nNOS activity in the brain occurs in the membrane fraction.[27–30]

The iNOS enzyme produces NO in 1000-fold greater quantities than the constitutive enzymes eNOS and nNOS in response to biologic inflammatory and immunologic factors, including cytokines, lipopolysaccharide, and interferon γ,[2,31] or possibly to counteract arteriosclerosis.[32] While inducible NO may kill invading organisms, the overproduction of NO from iNOS can be pathogenic and has been observed in many disorders, including septic shock, hemorrhagic shock, multiple sclerosis,

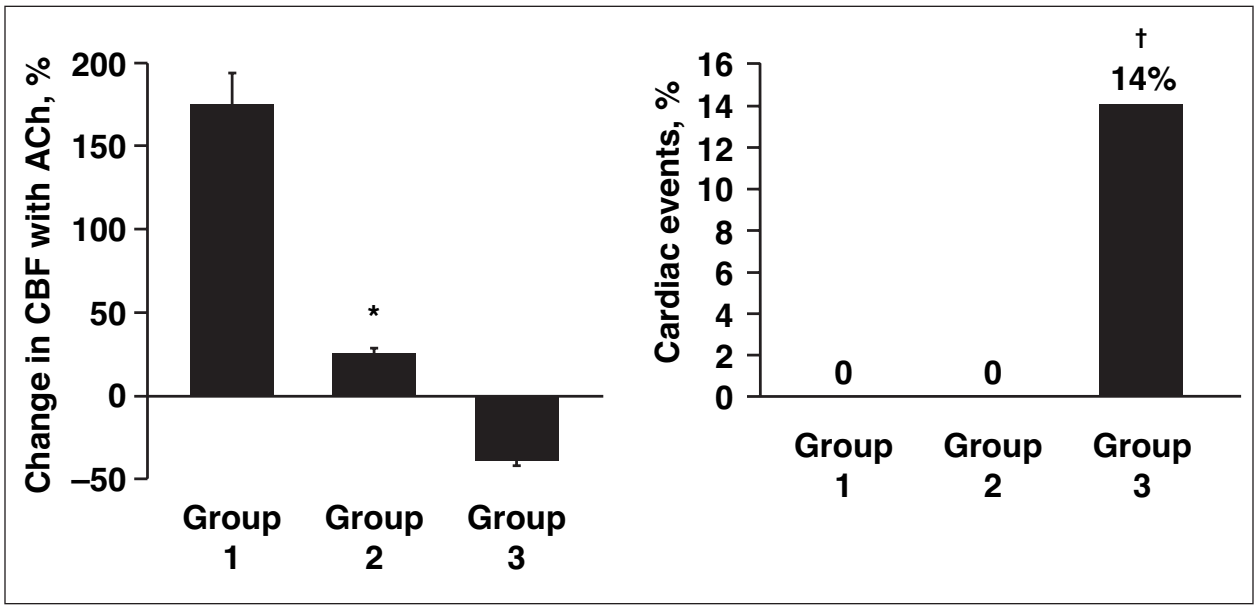

*Figure 1. In 157 patients with mild atherosclerosis followed for an average of 28 months, severe endothelial dysfunction at baseline (group 3; n=42), identified as reduced coronary blood flow (CBF) response to acetylcholine (ACh), was associated with a significantly increased risk for cardiovascular events compared with patients with normal (group 1; n=83) or mildly impaired (group 2; n=32) endothelial function. *P<.0001 vs groups 1 and 3. †P<.05 vs groups 1 and 2. Reproduced with permission from Al Suwaidi et al.*[51]

neurodegenerative diseases, rheumatoid arthritis, ulcerative colitis, and cancer.[2,31,33–35] Reduced eNOS and increased iNOS have been observed concomitantly in disease states.[35,36]

ENDOTHELIUM, NO, AND THE CV SYSTEM

The endothelium serves as a thromboresistant surface; a barrier to macromolecules; and a regulator of vascular tone, structure, and function by releasing multiple vasoactive substances, including NO, in response to physiologic stimuli.[3,37] Normal function of the endothelium relies on the balanced interaction of autocrine–paracrine relaxing and contracting factors within the vessel wall.[3,37] In response to hemodynamic shear stress, the endothelium continuously releases NO and up-regulates the gene that expresses NOS; NO provides vasodilation and maintains vascular tone.[37] In addition, the endothelium releases other interactive vasodilators.[38,39]

NO also exhibits antithrombotic and anti-inflammatory actions in the endothelium, including inhibition of vascular smooth muscle cell growth, platelet aggregation, cytokine expression, monocyte migration, leukocyte adhesion, and lipid oxidation.[38,40] NO down-regulates synthesis of angiotensin-converting enzyme (ACE) and angiotensin II type 1 receptors, thus suppressing the renin–angiotensin system, which promotes vasoconstriction and atherosclerotic processes and inhibits activity of the potent vasoconstrictor endothelin-1.[39,41] At the cellular level, NO regulates mitochondrial respiration and inhibits mitochondrial metabolism during cell stress, thereby reducing oxygen consumption and preventing the apoptosis related to oxidative damage.[1,42] Thus, NO plays a major role in maintaining endothelial homeostasis and function, regulating blood pressure (BP), and inhibiting atherosclerotic processes.[38,39,43]

The endothelial functions of NO also play a role in promoting systemic vascular and arterial relaxation and distensibility, thereby reducing cardiac preload and afterload, myocardial hypertrophy, and left ventricular dysfunction, and protecting target organs.[39,44–46] Furthermore, the vascular and cardioprotective functions of NO extend beyond the endothelium to involve neuronal NO and its role as a neuromodulator of the autonomic nervous system, maintaining vagal tone and suppressing sympathetic nervous system overactivity through both central and peripheral nervous system signaling.[47] Acting in the nucleus tractus solitarius, NO plays a significant role in regulating baroreflex function and cardiopulmonary reflex responses.[48]

Impaired NO in Endothelial Dysfunction

The importance of NO in promoting endothelial homeostasis is demonstrated by the association between impaired NO bioactivity and endothelial dysfunction, which is characterized by the imbalance of endothelium-derived vasoconstrictive and vasodilatory substances, with a shift toward greater vasoconstriction, inflammation, and thrombosis

formation.[3,49] Endothelial dysfunction is present during the early stages of atherosclerosis and is a significant predictor of CV morbidity and mortality (Figure 1).[3,40,44,50–52] This has prompted some researchers to conclude that the endothelium should be the focus of therapeutic strategies to reduce the risks of CV disease.[53] Reduced NO bioactivity is a major component of endothelial dysfunction.[3,36,54,55]

One proposed mechanism of impaired NO availability and endothelial dysfunction is oxidative stress, which occurs when pro-oxidant processes exceed the capacity of antioxidant mechanisms to maintain an appropriate balance. Oxidative stress is generated by increased production of reactive oxygen species, including superoxides, which are derived from xanthine oxidase, cyclooxygenase, and NAD[P]H oxidase. Reactive oxygen species react with NO, thereby decreasing its bioactivity.[10,49] Some well-known CV risk factors such as hyperlipidemia, hypertension, diabetes, and cigarette smoking are associated with oxidative stress.[49,54,56]

Impaired NO and Hypertension

Endothelial dysfunction is often described in individuals with hypertension.[3] Multiple studies of flow-mediated dilatation of the brachial artery, usually examined by venous occlusion plethysmography of forearm blood flow, have shown that patients with hypertension exhibit blunted arterial vasodilation in response to endothelium-dependent vasodilators, such as ACh, while vasodilatory response to endothelium-independent vasodilators, such as sodium nitroprusside, is preserved (Figure 2).[43,57,58] Moreover, inhibition of NO with infusion of N^G-monomethyl-L-arginine has significantly increased BP and total peripheral artery resistance (Figure 3).[59] It is unclear, however, whether endothelial dysfunction (with NO impairment) is a cause or consequence of increased BP.[3] Regardless of the mechanism, endothelial dysfunction may increase BP, and CV risk is positively and significantly correlated with the severity of endothelial dysfunction in hypertensive patients.[3,44,50]

Impaired NO and CV Disease

Impairment of NO bioactivity has been viewed as a CV risk factor due to its strong association with endothelial dysfunction[3,60]; however, studies have also identified the CV risks of reduced NO bioactivity per se. Studies have found, for example, that inhibition of NO with N^G-monomethyl-L-arginine increases arterial stiffness.[45,61] Moreover, impaired platelet production of NO was shown to be a significant, independent risk factor, and a stronger predictor than atherosclerosis, for acute coronary syndromes in patients undergoing coronary angiography.[62] Another study showed that release of NO, as measured with a porphyrinic microsensor in atherosclerotic human carotid arteries and normal mammary arteries, was markedly reduced in the atherosclerotic arteries compared with normal arteries.[55] In patients with coronary heart disease (CHD), elevated C-reactive protein level, an inflammatory marker, was shown to be significantly and independently associated with impaired NO bioavailability as measured by forearm blood flow vasodilatory response to N^G-monomethyl-L-arginine; these findings further support the hypothesis that oxidative stress and inflammation are major causes of impaired NO.[63]

Impaired NO in the Cardiometabolic Syndrome

Experimental data indicate that glucose stimulates islet activities of both constitutive and inducible forms of NOS, and NO may serve as a negative feedback inhibitor of inappropriate insulin release at hyperglycemic glucose levels.[64] Other preliminary data indicate that NO may stimulate insulin release.[65] Clinical studies have shown that the arterial vasodilating effect of insulin in skeletal muscle, a primary mechanism of insulin sensitivity, is dependent on endothelial NO release.[14,66] Overall, NO appears necessary for regulation of insulin secretion, normal β-cell function, and insulin sensitivity.[14,67]

Decreased NO bioactivity and endothelial dysfunction are associated with insulin resistance and diabetes mellitus.[14,68,69] Because the arterial vasodilating effect of insulin in skeletal muscle, which is a primary mechanism of insulin sensitivity, is dependent on endothelial NO release, NO impairment would theoretically reduce insulin sensitivity.[14,66] It is unclear, however, whether impaired insulin action causes decreased NO bioactivity, or vice versa, or whether both defects arise from a common biologic source.[66] One proposed pathogenic mechanism supporting this association is a genetic and/or acquired defect of NO, leading to the syndrome of reduced insulin-mediated vasodilation, insulin resistance, and diabetes.[14,70]

NON-CV FUNCTIONS OF NO

Central and Peripheral Nervous System

In the brain, NO is a multifunctional messenger molecule involved in processes of synaptic plasticity, learning and memory, and possibly aging.[71,72] The antioxidant and antiapoptotic effects of NO may protect against neurodegeneration,[72] and vascular

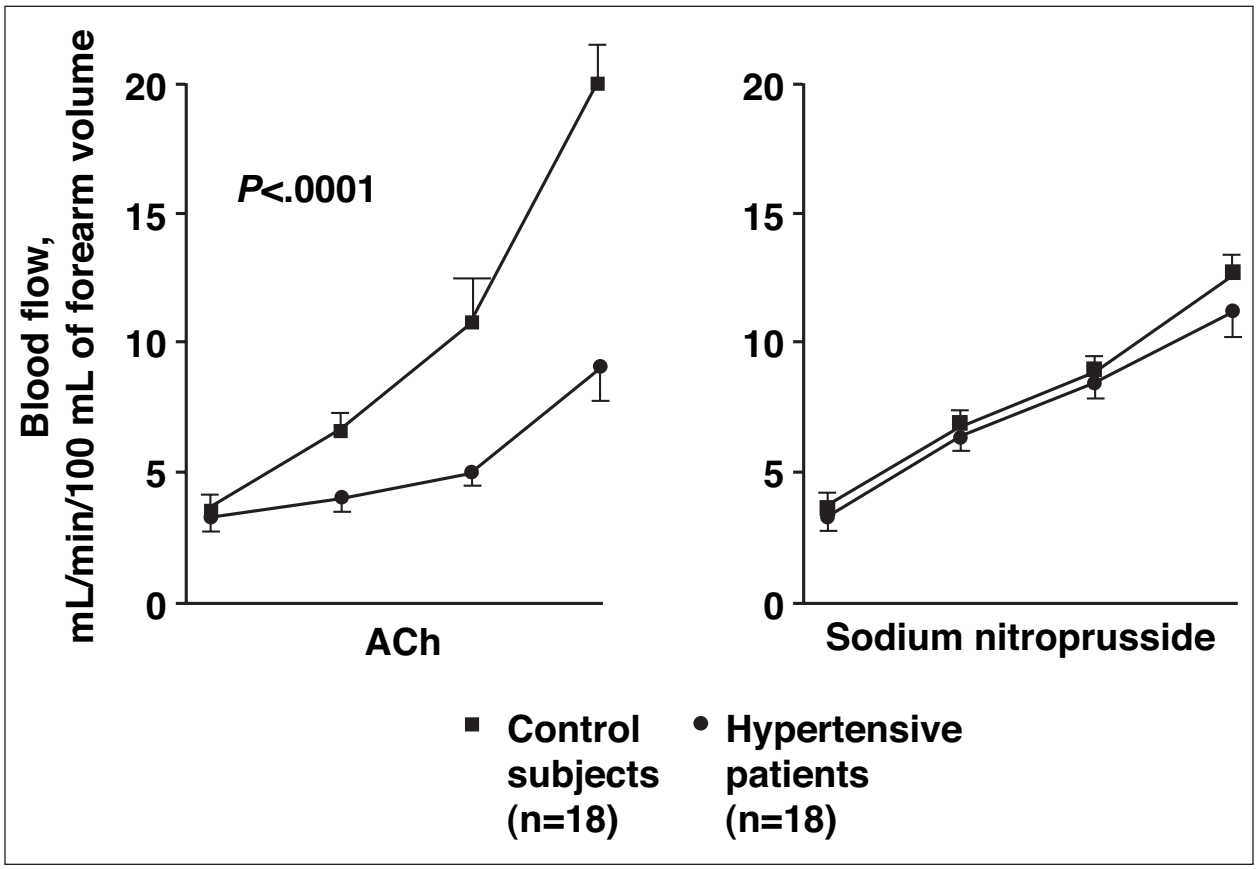

Figure 2. The effects of infusion of acetylcholine (ACh), an endothelium-dependent vasodilator, and sodium nitroprusside, a direct endothelium-independent vasodilator, on forearm blood flow (FBF) in the brachial artery were compared in 18 men and women with hypertension and 18 normotensive controls. As measured by strain-gauge plethysmography, FBF ACh was significantly reduced in the hypertensive patients compared with the control patients (P<.0001), but no significant difference in FBF was observed between hypertensive and control patients in those infused with sodium nitroprusside. Reproduced with permission from Panza et al.[57]

NO may promote cerebral perfusion, which improves memory function.[73] A study in an aging rat model of chronic brain hypoperfusion, which mimics human mild cognitive impairment, found that selective inhibition of eNOS impaired spatial memory function, suggesting that this brain function is dependent on NO-regulated maintenance of cerebral blood flow and microvessel tone.[73] The effects of NO in neurodegenerative diseases are highly controversial, however, with evidence that NO may assume pathogenic as well as protective roles.[15,72] In the normal brain, for example, NO inhibits precursor brain cell proliferation; however, after brain damage, excessive NO production may promote pathologic neurogenesis.[15,74]

In the peripheral nervous system, NO serves as a gastrointestinal neurotransmitter, mediating relaxation and tone of the stomach, colon, and internal anal sphincter.[2] Neuronal NO in the brain may also mediate the inhibition of gastric acid secretion, a stress-induced defense mechanism.[27,30] In skeletal muscle, NO mediates excitation-contraction coupling, mitochondrial energy production, glucose metabolism, and autoregulation of blood flow.[75] In addition, neuronal NO contributes to the regulation of renal function[76] and NO performs an important renoprotective role in opposing the effects of chronic renal renin–angiotensin system overactivation, which contributes to renal hypertension and injury.[39] Inhibition of NO has also been associated with accelerated hypertensive renal disease in rats.[76]

Reproductive System

Male Reproductive System. In men, NO regulates relaxation of smooth muscle cells of the corpus cavernosum and is a principal mediator of penile erection, speculatively through a combination of neuronal, neurohormonal, and vascular effects.[2,77] NO primarily acts as a neurotransmitter of erogenic stimuli, which diffuses to vascular and trabecular smooth muscle in the penis, thereby increasing blood flow and promoting erectile tumescence.[77] Multiple animal studies have shown that decreased NOS is correlated with erectile dysfunction related

to aging and diabetes.[78–80] Moreover, in clinical studies, reduced flow-mediated dilation of the cavernous arteries[81] and of the brachial artery[82] were significantly correlated with erectile dysfunction.

Female Reproductive System. In women, NO increases during pregnancy and contributes to the reduction of peripheral vascular resistance through vascular smooth muscle relaxation. Although smooth muscle relaxation is observed in normal pregnancy, the role of NO bioactivity in this process appears to vary by species, vessel size, and vascular bed.[13,83] Additional data suggest that NO regulates uterine quiescence, cervical ripening, and labor.[84–86] Levels of NO in the uterus are elevated during pregnancy and significantly decreased during labor, whereas they are low in the cervix during pregnancy and increase dramatically before and during labor.[84–86] Production of NO in the uterus during pregnancy may be elevated to promote uterine relaxation and inhibit contractility, and the decrease of NO at term may help signal or promote labor.[84] Similarly, NO levels may be suppressed in the cervix during pregnancy to inhibit preterm labor, and may increase at term to enhance cervical ripening.[17]

Reduced NO may play a role in preeclampsia.[13] Several studies in isolated human resistance arteries from pregnant women in vitro demonstrated that flow-mediated, endothelium/NO-dependent vasodilatory responses were reduced in women with preeclampsia, compared with normotensive women.[87–89] Reduced flow-mediated dilation of the brachial artery has also been observed in vivo in pregnant women with preeclampsia, compared with normotensive pregnant women,[90] and in pregnant women tested at 23–25 weeks' gestation who later developed preeclampsia, suggesting that endothelial dysfunction precedes preeclampsia.[91] Other clinical studies, however, have failed to show an association between impaired NO and preeclampsia.[92,93]

Respiratory System

NO is produced in the respiratory tract by multiple cell types, including epithelial cells, airway neurons, inflammatory cells, and vascular endothelial cells.[5] In the healthy lung, NO regulates pulmonary vascular reactivity and induces airway dilation in response to hypoxia-induced vasoconstriction.[5,11] NO may be an important regulator of pulmonary vascular homeostasis because of its endothelial antithrombotic and anti-inflammatory effects, and neuronal NO may also modulate bronchomotor tone.[11] In contrast, excessive production of NO

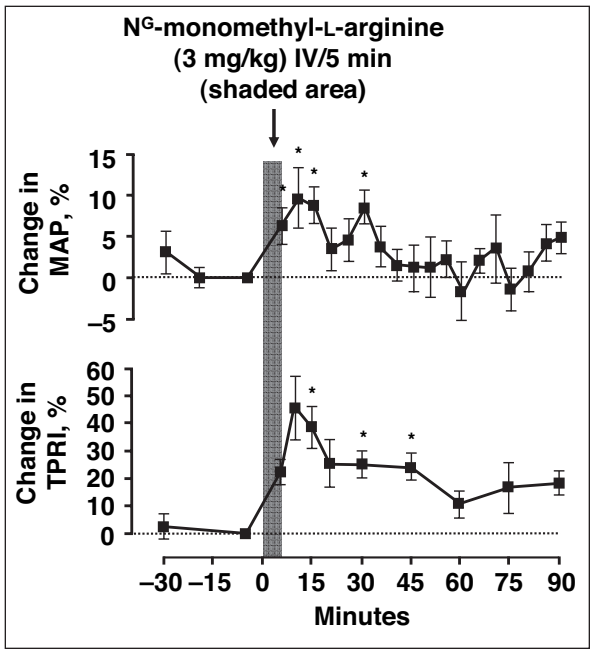

Figure 3. Intravenous (IV) infusion of 3 mg/kg N^G-monomethyl-L-arginine, an inhibitor of nitric oxide synthase, significantly increased mean arterial pressure (MAP) by 10% (P<.05) and increased total peripheral resistance index (TPRI) by 46%, compared with saline placebo (P<.05), in 8 healthy subjects. Reproduced with permission from Haynes et al.[59]

induced by poorly controlled pulmonary inflammation is associated with asthma.[5,11] Overall, balanced production of NO appears to be essential for normal functioning of the lung.[11]

Reduced expression of NO in the vascular endothelium of pulmonary arteries, as determined by histochemical and immunohistochemical analysis, has been significantly associated with primary and secondary pulmonary hypertension. The severity of pulmonary hypertension is proportional to the extent of NO reduction, although the pathophysiologic mechanism of this association is unclear.[11,94] Reduced endothelium-dependent vasodilation also has been observed in patients with chronic obstructive pulmonary disease and cystic fibrosis, although the evidence of these associations is inconsistent.[11,95] In addition, a clinical study found that the immunohistochemical expression of eNOS in the arterial endothelium and protein content of lung tissue in smokers were lower than in control nonsmokers, suggesting that NO impairment from smoking is a pathogenic factor in respiratory disease associated with tobacco.[96] Preliminary data also suggest that airway hyper-responsiveness, seen in asthma, may be associated with reduced endogenous NO in the lungs.[5] Paradoxically, however, asthma is associated with increased expression of

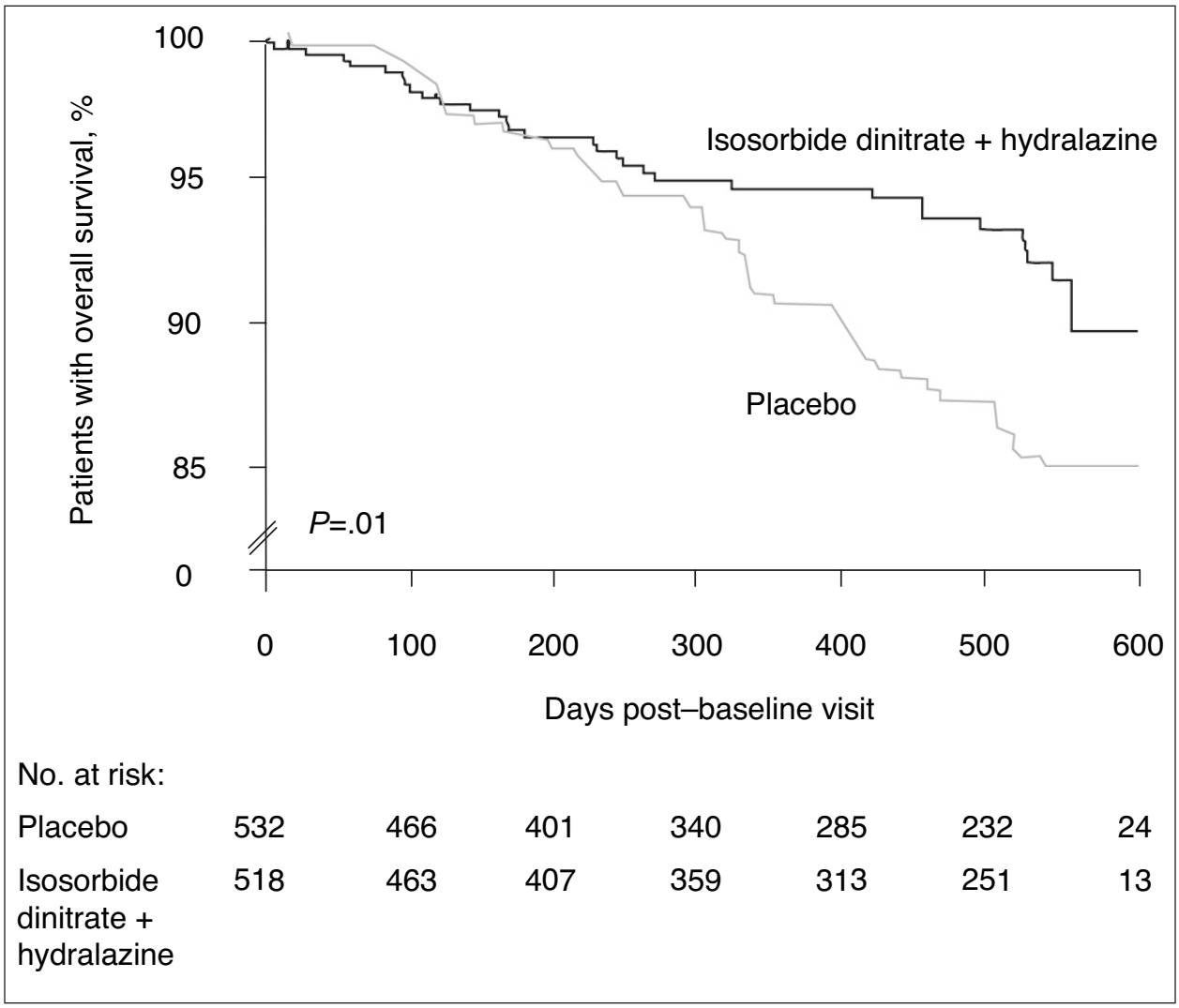

*Figure 4. In the African-American Heart Failure Trial (A-HeFT), conducted in 1050 black patients with New York Heart Association class III or IV heart failure, treatment with a fixed dose of isosorbide dinitrate, a nitric oxide donor, plus hydralazine, when added to standard heart failure therapies, significantly reduced mortality by 43% (*P=.01*) compared with placebo. Reproduced with permission from Taylor et al.*[33]

iNOS and elevated levels of inducible NO, which have proinflammatory effects.[5]

Bone Formation

Both constitutive and inducible forms of NO have been observed in a variety of bone cells, where they perform multiple functions.[6,97] At low levels, endothelial and neuronal constitutive NO mediate normal proliferation of bone-forming osteoblasts and inhibit the activity of bone-resorbing osteoclasts, promoting normal bone turnover and preventing osteoporosis.[97,98] A study in animal bone models found that NOS inhibition with *N*-nitro-L-arginine methyl ester and aminoguanidine increased osteoclast resorptive activity, dramatically increasing the number and size of bone pits, compared with control bone cultures in vitro, and caused increased resorptive activity and a loss of bone density in an in vivo rat model of osteoporosis.[99] High levels of inducible NO, however, promoted by inflammatory states including rheumatoid arthritis or osteomyelitis, may exacerbate the osteoporotic processes caused by those conditions.[6,97,98] While controlled levels of constitutive NO are essential for normal bone growth, excessive inducible NO is pathogenic in osteoporosis.[6,97]

THERAPEUTIC MODULATION OF NO

As the effects of impaired NO production and release have become increasingly defined in the pathophysiology of CV disease states, therapeutic strategies seeking to increase the availability of NO have been rapidly emerging.[16,17,100] These strategies include agents that donate or release NO,

stimulate endogenous production of NO, or protect NO from oxidative stress and inactivation.

NO Donors

Chemicals that release NO are being widely investigated for their potential in diverse therapeutic areas.[16,23,101] Nitrates such as nitroglycerin and nitroprusside provide acute vasodilatory relief in acute CV conditions such as angina, myocardial infarction, and hypertensive emergencies. The usefulness of these drugs is considered limited, however, because of adverse effects and the occurrence of nitrate tolerance with chronic administration.[23]

The African-American Heart Failure Trial (A-HeFT),[33] conducted in 1050 black patients, supported the use of organic nitrate therapy to improve CV outcomes in patients with New York Heart Association class III or IV heart failure. Treatment with the NO donor isosorbide dinitrate plus hydralazine, added to standard heart failure therapies including diuretics, ACE inhibitors, and β-blockers, achieved reductions of 43% for overall mortality (*P*=.01) and 33% for heart failure hospitalizations (*P*=.001), as well as improved quality-of-life scores (*P*=.02), compared with placebo (Figure 4). These results, which prompted early termination of the trial after a mean follow-up of 10 months, provide the first substantial data indicating that NO modulation can improve CV outcomes in heart failure.[33]

The nitrate nicorandil has demonstrated not only relief of angina pectoris but also significant reductions of multiple CV disease outcomes in major randomized controlled clinical trials.[102,103] In the Impact of Nicorandil in Angina (IONA) study[103] (N=5126), addition of nicorandil to standard antianginal therapy significantly reduced the primary composite end point of CHD death, nonfatal myocardial infarction, or unplanned hospitalization for angina pectoris (*P*=.01); acute coronary syndromes (*P*=.03); and all CV disease events (*P*=.03), compared with placebo.

In other disease areas, data from the Study of Osteoporotic Fractures[104] showed that intermittent use of nitrate supplements in 317 elderly women was associated with substantially increased bone mineral density of the hip and heel, compared with nonusers. Furthermore, topical nitroglycerine ointment was as effective as estrogen therapy in preventing bone loss in women who had undergone oophorectomy.[105] Several novel nitrates are also being investigated for their potential effects in providing neuroprotection in neurodegenerative diseases, including disorders such as dementia and Alzheimer's disease.[72]

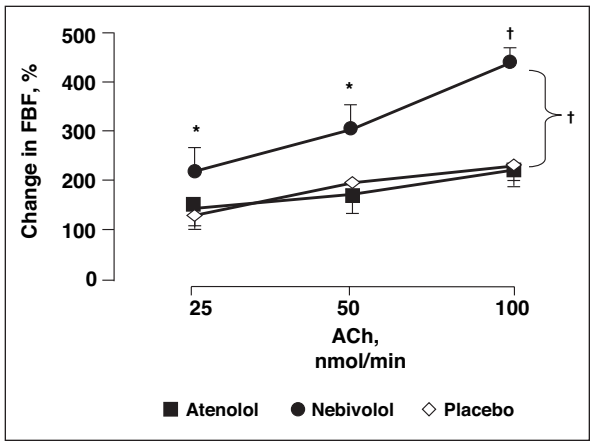

*Figure 5. A double-blind, randomized, 8-week, crossover study in 12 hypertensive patients showed that nebivolol 5 mg + bendrofluazide 2.5 mg once daily significantly increased vasodilatory response to acetylcholine (ACh), as indicated by forearm blood flow (FBF), compared with baseline, while atenolol 50 mg + bendrofluazide 2.5 mg once daily and placebo had no effect on the vasodilatory response to ACh. *P<.05. †P<.001, compared with baseline. Reproduced with permission from Tzemos et al.[111]*

Inhaled NO has been used extensively for more than a decade in neonates and children for treatment of cardiorespiratory failure[106] and in adults for acute respiratory failure and pulmonary hypertension in critical care and perioperative settings.[107] Inhaled NO in adults has reversed right ventricular dysfunction secondary to hypoxia and may alleviate other cardiopulmonary disorders, including idiopathic fibrosis.[11] In addition, *S*-nitrosothiols are a new class of NO donor drugs, which include molecules derived from the reactions of endogenous NO with thiol groups; this class of drugs may avoid some of the drawbacks of the classic organic nitrates, and may enhance the anti-inflammatory and antineoplastic functions of NO.[23]

Hybrid NO Donors. New hybrid NO donors are being developed, including NO-releasing nonsteroidal anti-inflammatory drugs (NO–NSAIDs), such as NO–aspirin, NO–naproxen, and NO–ibuprofen.[16] These drugs are designed in part to avert the gastric injuries caused by NSAIDs; release of NO in animals has protected the stomach lining, perhaps by prevention of leukocyte adhesion on the endothelium, maintenance of mucosal blood flow, or stimulation of mucus secretion.[16,27,101] Data from multiple animal studies suggest that NO–NSAIDs provide enhanced efficacy and safety, compared with NSAIDs alone, in various inflammatory conditions, including rheumatoid arthritis, irritable bowel syndrome, colitis, and central nervous system inflammation.[16]

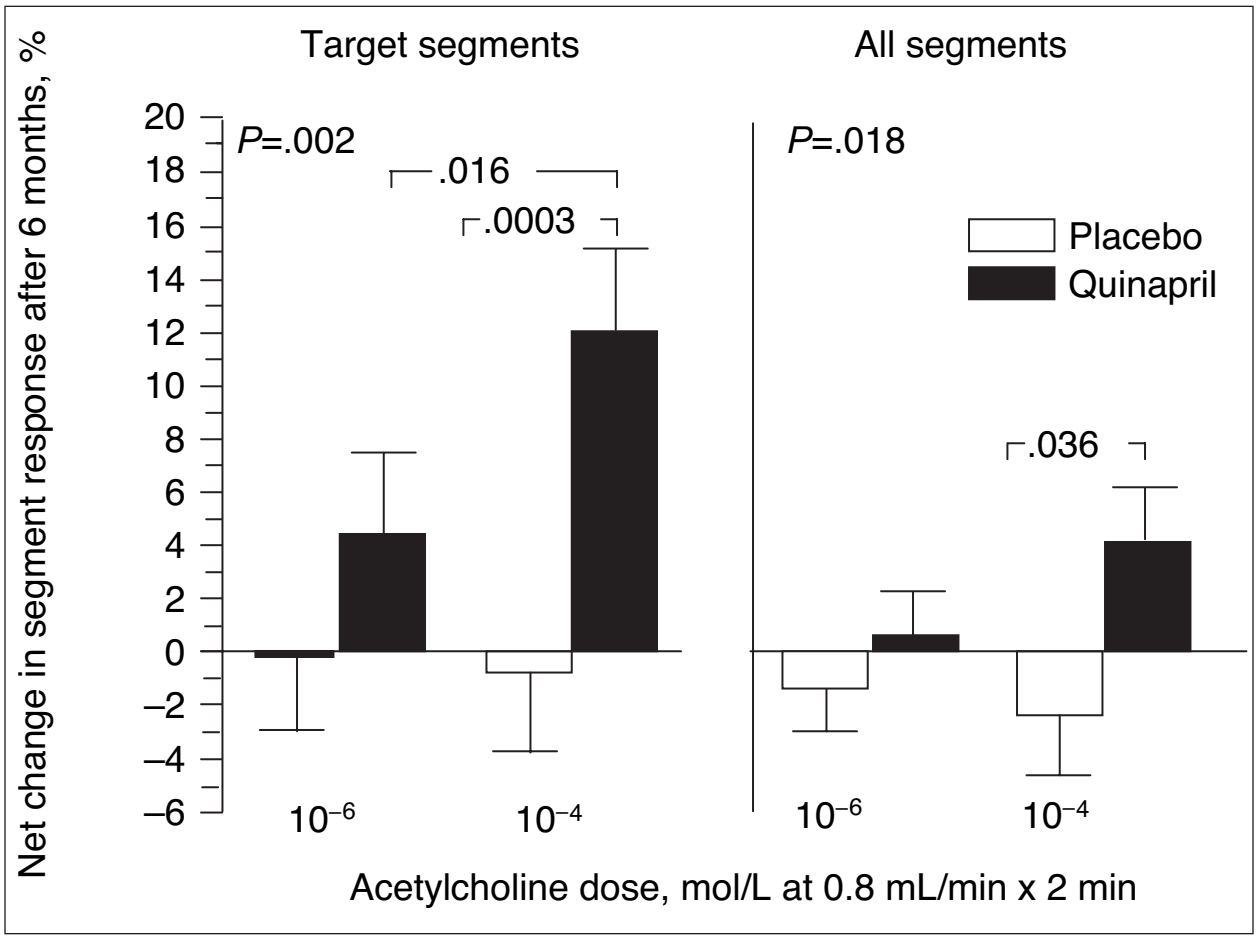

Figure 6. In the Trial on Reversing Endothelial Dysfunction (TREND), a double-blind, randomized, placebo-controlled trial, treatment with the angiotensin-converting enzyme inhibitor quinapril for 6 months in 105 normotensive patients with coronary atherosclerosis significantly improved coronary artery vasomotor function as determined by the net percentage change in the acetylcholine-provoked vasoconstriction response, compared with placebo, in target segments and in all segments. Reproduced with permission from Mancini et al.[116]

NO Stimulants

Several therapies used to treat CV disease and hypertension have increased NO availability.[100] These include antihypertensive drugs, such as β-blockers, inhibitors of chronic renin–angiotensin system activation, calcium channel blockers (CCBs), and 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins) and nonpharmacologic approaches.[100,108,109]

β-Blockers. The cardioselective β-blocker nebivolol has demonstrated significant improvement in endothelium/NO-dependent vasodilation in healthy volunteers and patients with hypertension, compared with either placebo or atenolol, due to its ability to increase NO release (Figure 5).[110,111] The β-blocker carvedilol may also improve endothelial function in high-risk patients, possibly due to antioxidant activity.[112,113] Other β-blockers that may improve endothelial/NO function, based on preliminary evidence, include bopindolol and celiprolol.[114] Interestingly, a clinical comparison study in 550 hypertensive patients found that nebivolol and celiprolol significantly reduced plasma levels of all 3 markers of thrombosis measured (homocystine, fibrinogen, and plasminogen activator inhibitor 1), thus suggesting promotion of antioxidant NO bioactivity, whereas carvedilol had no significant effect on any of these measures.[115]

Renin–Angiotensin System Modulating Drugs. Considerable data indicate that ACE inhibitors significantly improve endothelium-dependent vasodilation and reverse endothelial dysfunction in normotensive patients and in hypertensive and CV disease patients (Figure 6).[100,116–119] In addition, ACE inhibitors have demonstrated improvement of endothelial function in high-risk hypertensive patients with diabetes mellitus and nephropathy[120,121] and hypercholesterolemia and CHD.[122,123] These agents are hypothesized to promote NO bioactivity through preservation of

bradykinin and stimulation of bradykinin β_1 or β_2 receptors, which promote NO release, and through antioxidant activity via suppression of angiotensin II, although the precise mechanisms of these effects remain unclear.[100,124,125]

Angiotensin II receptor blockers (ARBs) may also promote NO bioactivity, possibly through suppression of angiotensin II, leading to reduction of oxidative stress factors, and through activation of the angiotensin II type 2 receptor, which stimulates the bradykinin/β_2 receptor to release NO.[126,127] In clinical studies, ARBs have demonstrated significant improvement of endothelial function and apparent promotion of NO bioactivity in patients with hypertension, hypercholesterolemia, and CV disease.[119,127–129] Other trials, however, have shown no effect of ARBs on endothelial function.[118,130,131]

Calcium Channel Blockers. Substantial data also show that several CCBs may significantly improve endothelial function and restore NO bioactivity in various vascular beds, primarily through antioxidant activity,[100,132–134] although other studies have shown no improvement in endothelial function with CCBs in patients with hypertension and CV disease.[100,130,135]

Statins. Statins have been shown to improve various indices of endothelial function, including increased coronary blood flow, reduced levels of adhesion molecules, and stimulation or restoration of eNOS.[136,137] In addition, statins inhibit the activity of angiotensin II and endothelin-1, and reduce superoxide production and inflammatory processes associated with atherosclerosis, thus increasing NO in endothelial cells.[136,137] In clinical trials, several statins significantly improved endothelium-dependent vasodilation in patients with hypercholesterolemia and other high-risk populations.[138–141] Immunoassay studies found that statins up-regulate the synthesis and bioactivity of endothelial NO through multiple pathways, including reduction of superoxide anion formation and decrease of caveolin-1, a vascular inhibitor of eNOS activity (Figure 7).[137,142,143]

L-Arginine. The sole substrate for NOS, L-arginine, is an amino acid produced endogenously but mainly derived in humans through common dietary sources and as an oral supplement.[144] Clinical studies have been inconsistent. A review of 17 small clinical studies of oral arginine supplement treatment found 12 studies showing reduced thrombotic activity and improved endothelium/NO-dependent vasodilation and 5 studies showing no CV benefit.[145] Two studies in women with preeclampsia showed that L-arginine treatment significantly reduced BP, possibly through increased NO bioactivity.[146,147] Another clinical study, however, found that oral L-arginine treatment for 2 days did not reduce diastolic BP in preeclamptic women.[148]

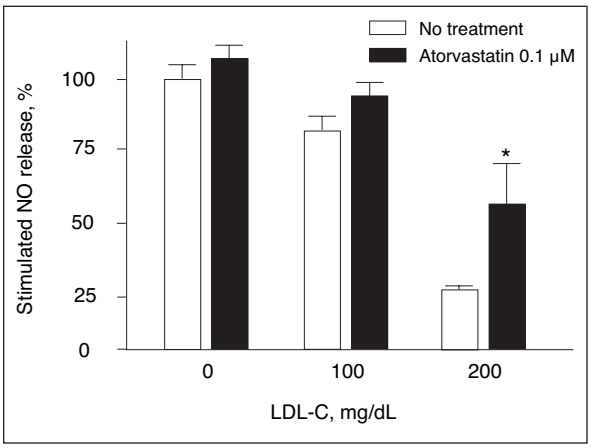

*Figure 7. Three samples of intact endothelial cells were preincubated with or without low-density lipoprotein cholesterol (LDL-C), 100 mg/dL or 200 mg/dL, and the statin atorvastatin, 0.1 μmol/L, and then exposed to the calcium ionophore A23187, 5 μmol/L, a receptor-independent agonist known to promote the binding of Ca^{2+}-activated calmodulin to endothelial nitric oxide synthase (eNOS). Exposure to LDL-C without cotreatment with atorvastatin (open bars) substantially decreased nitric oxide (NO) release, while atorvastatin treatment (solid bars) significantly increased NO release from 5% in cells not exposed to LDL-C to 17% and 107% in cells exposed to LDL-C 100 mg/dL and 200 mg/dL, respectively. The greater effect of atorvastatin with higher exposure to LDL-C is believed to derive from statin-induced reduction of LDL-C and caveolin-1, which rises in concentration with increasing LDL-C and potently inhibits eNOS function and NO release (see Figure 1). *P<.01 vs no treatment with atorvastatin. Reproduced with permission from Feron et al.[143]*

Nonpharmacologic Stimulants of NO

Several nonpharmacologic therapies used in the prevention of CV disease may promote increased NO production and release. Moderate and intense exercise significantly improved endothelium/NO-dependent vasodilation in healthy and hypertensive subjects[149,150] and in patients with stable CHD.[151] In addition, studies have found that treatment with red wine from France in human endothelial cells in vitro resulted in significant increases in eNOS expression and NO release.[152,153] Incubation of platelets with purple grape juice and purple grape juice–derived flavonoids—substances that may account for the effects of red wine on NO—also decreased platelet aggregation, increased platelet-derived NO, and decreased

superoxide production.[154] Other sources of antioxidant flavonoids, such as black tea and cocoa, also improved endothelial function.[155,156] Ethanol also inhibits the activity of iNOS, an effect that may explain some of the CV benefits of moderate alcohol consumption.[157]

Some studies suggest that nutritional supplements such as vitamins C and E, omega-3 fatty acids, or folate may improve endothelial and NO function in patients with CV risk factors or disease, although these data are generally inconsistent.[109] A low-calorie diet was also shown to significantly improve endothelium/NO-dependent vasodilation in obese patients with hypertension.[158]

CONCLUSIONS

NO is a signaling molecule fundamental to a wide array of physiologic processes. Impaired NO bioactivity has been strongly associated with endothelial dysfunction and CV disease and is implicated to varying degrees in multiple disorders ranging from osteoporosis to erectile dysfunction. Various approaches to the therapeutic modulation of NO have been investigated, including the use of NO donors, antihypertensive agents, statins, L-arginine, and nonpharmacologic CV therapies. Many of these therapies appear to significantly improve NO bioactivity, suggesting potential avenues for treatment. Data from the recent A-HeFT trial showed that use of NO-donor therapy significantly reduced morbidity and mortality in black patients with heart failure, providing the first substantial evidence that NO modulation may affect CV outcomes. Further data on the effects of NO modulation on outcomes in a range of disorders should help clarify the usefulness of this therapeutic approach.

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