

# Vasoprotective Effects of Nitric Oxide in Atherosclerosis

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## *Vasoprotective Effects of Nitric Oxide In Atherosclerosis* Summary

The endothelium plays a crucial role in the process of atherosclerotic disease by means of its regulatory functions on the vasculature, such as control of vasomotor tone, local hemostasis and proliferative processes. One of the most important substances released by the endothelium is nitric oxide (NO), which acts as a vasodilator, and inhibits the proliferation of vascular smooth muscle cells, the aggregation of platelets and the infiltration of inflammatory cells. A reduction in NO production or activity has been proposed as a major mechanism of endothelial dysfunction and a contributor to atherosclerosis. Since endothelial dysfunction is considered an early marker for atherosclerosis and can be detected before structural changes in the vascular wall, an impairment of NO bioactivity or synthesis will reduce its braking effect on processes involved in atherogenesis. This review briefly describes the vasoprotective actions of NO, its role in the pathogenesis of atherosclerosis, and the novel therapeutic strategies improving endothelial dysfunction.

**Key Words** Endothelium, endothelial dysfunction, nitric oxide, atherosclerosis

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## *Aterosklerozda Nitrik Oksidin Vazoprotektif Etkileri* Özet

Endotelyum, proliferatif süreç, lokal homeostaz ve vazomotor tonusun kontrolü gibi düzenleyici fonksiyonları aracılığı ile aterosklerozda önemli bir rol oynamaktadır. Endotelyum tarafından salınan en önemli maddelerden biri olan nitrik oksid (NO), vasküler düz kas hücrelerinin profilasyonunu, trombosit agregasyonunu ve inflamatuvar hücrelerin infiltrasyonunu inhibe eden bir vazodilatör olarak etki göstermektedir. Endotel tabakasının fonksiyonunun bozulması aterosklerozun erken dönem belirticidir ve damar duvarında yapısal değişiklikler oluşmadan önce belirlenebilir. NO üretimi veya biyoaktivitesindeki bir eksiklik, nitrik oksidin ateroskleroz süreci üzerindeki engelleyici etkisini azaltacaktır. Bu makale, kısaca, NO'nun vazoprotektif etkilerini, ateroskleroz patogeneziindeki rolünü ve endotel disfonksiyonunun düzeltilmesinde etkili olan yeni terapötik stratejileri tanımlamaktadır.

**Anahtar Kelimeler:** Endotelyum, endoteliyal disfonksiyon, nitrik oksid, ateroskleroz

## INTRODUCTION

The endothelium is involved in a wide variety of physiological and pathological processes. Endothelial cells have a major role in the regulation of vascular tone by producing a number of vasodilator and vasoconstrictor substances<sup>1-4</sup>. Among the vasodilatory agents produced by the endothelium are nitric oxide (NO), prostacylin, bradykinin, endothelium-derived hyperpolarizing factor and C-type natriuretic peptide.

Vasoconstrictors include endothelin, superoxide anion, endothelium-derived constricting factor that is poorly characterized, locally produced angiotensin II, and thromboxane<sup>5</sup>. The role of the endothelium is not confined to the regulation of vascular tone and vasomotor function but extends to the regulation of inflammation, platelet activation, and thrombosis.

The endothelium maintains the balance between vasodilation and vasoconstriction, inhibition and

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stimulation of smooth muscle cell proliferation and migration, and fibrinolysis<sup>6,7</sup>. When the balance is impaired, endothelial dysfunction occurs, resulting in atherosclerosis, hypertension and related diseases. Endothelial dysfunction is considered an early marker for atherosclerosis<sup>6</sup>. Disruptions in the functional integrity of the vascular endothelium plays an integral role in all stages of atherogenesis, ranging from lesion initiation to plaque rupture. NO is a principal factor involved in the antiatherosclerotic properties of the endothelium<sup>1,2,8</sup>. Major risk factors for atherosclerotic vascular disease, such as hypercholesterolemia, diabetes, hypertension, and smoking, have been associated with impaired NO activity. Physical or biochemical injury to the endothelium impairs production of homeostatic mediators of vascular health, such as NO, resulting in an intima that is characterized by enhanced thrombus formation, aberrant vessel tone, and dysregulated vascular smooth muscle cell growth. In this review, the importance of NO as a mediator of vascular function, the role of NO in the pathogenesis of atherosclerosis and the novel therapeutic strategies will be discussed.

## BIOLOGICAL CHARACTERISTICS OF NO

In 1980, Furchgott and Zawadzki<sup>1</sup> discovered that the endothelium releases a factor that relaxes the underlying vascular smooth muscle. They termed this substance endothelium-derived relaxing factor (EDRF). Seven years later, Palmer et al.<sup>2</sup> and Ignarro et al.<sup>9</sup> independently showed that EDRF was equivalent to NO. NO is a highly diffusible inorganic radical gas with a very short half-life (3-6 sec.). NO is synthesized in mammals by the action of a family of enzymes called nitric oxide synthases (NOS), which convert the amino acid L-arginine into NO and another amino acid, L-citrulline. To date, three isoforms of nitric oxide synthase have been identified: an endothelial type (eNOS), a neuronal type (nNOS), and an inducible type (iNOS). eNOS and nNOS are two forms of constitutive Ca<sup>++</sup>- and calmodulin-dependent NO synthases, i.e. requiring calcium ions and calmodulin for their activation<sup>10</sup>. The eNOS is expressed constitutively in endothelial cells and synthesizes the NO needed for regulation of blood

pressure. In endothelial cells, this enzyme is bound to cell membrane-associated caveolae. While the enzymatic activity of eNOS is stimulated by Ca<sup>++</sup>/calmodulin, it is inhibited by caveolin-1, a membrane protein of caveolae<sup>11</sup>. The inducible form of NOS enzyme is found in vascular smooth muscle, endothelium, and macrophages<sup>12,13</sup>. The enzyme, which is calcium-independent, produces large amounts of NO; it is induced by cytokines such as endotoxin, interleukin-1 $\beta$ , and tumor necrosis factor (TNF), and is activated in inflammatory processes and endotoxin shock<sup>14,15</sup>. Neuronal NOS is thought to be most widely distributed among the isoforms, and in addition to its neural functions, it regulates the secretion in kidney, epithelial cells of the lung, stomach, pancreatic islets, and uterus as well as the non-vascular smooth muscle functions<sup>13</sup>. These enzymes, all require several cofactors for proper function, including tetrahydrobiopterin (H<sub>4</sub>B), nicotinamide adenine dinucleotide phosphate (NADPH), flavin-adenine dinucleotide (FAD), and flavin-mononucleotide (FMN).

NO is a very lipophilic compound and thus does not require a cell receptor to mediate its action on vascular smooth muscle. NO activates the enzyme soluble guanylate cyclase (sGS) to produce the second messenger cyclic guanosine monophosphate (cGMP). Heme is required for the activation of GS by NO. Without heme, NO has little activity on sGS<sup>16</sup>. Thus, the "receptor" for NO in vascular smooth muscle appears to be the heme component of sGS. NO and its receptor, sGS, are emerging as key mediators coordinating adenosine 5'-triphosphate (ATP) supply and demand. The mechanism coupling this pathway with metabolic and energetic signaling remains undefined. Ruiz-Stewart et al.<sup>17</sup> have demonstrated that sGS is a nucleotide sensor whose responsiveness to NO is regulated by ATP. It has been observed that NO stimulates adenosine 5'-diphosphate (ADP) ribosylation of many soluble and membrane-bound proteins including G-proteins in vascular smooth muscles. This leads to the activation of adenylate cyclase activity and inhibition of phospholipase-C activity leading to vasodilation<sup>18</sup>. They hypothesized that in a hypertensive state, the chronically decreased levels of NO lead to decreased ribosylation of G-

proteins leading to vasoconstriction. However, there is a marked cGMP-independent component of NO-mediated relaxation in almost all vessels studied to date, although the activation of the sGS is thought to be the main pathway by which NO induces relaxation<sup>19</sup>.

## POTENTIALLY VASOPROTECTIVE MECHANISMS OF NITRIC OXIDE IN ATHEROSCLEROSIS

### *Vasodilator effects of NO*

NO is the key endothelial-derived relaxing factor that diffuses from the endothelial cells to the vascular smooth muscle and increases cGMP, causing relaxation of the artery. The vasorelaxant effect of NO has been well documented in vivo and in vitro<sup>20-22</sup>. The endothelium-dependent vasodilator response may serve as surrogate for the bioavailability of NO. A well-established technique for the evaluation of coronary endothelial function is the assessment of the change in epicardial coronary artery diameter in response to graded concentrations of intracoronary acetylcholine (ACh) using quantitative coronary angiography<sup>23,24</sup>. Similarly, microvascular endothelial function of the coronary circulation can be assessed by Doppler flow velocity measurements in response to ACh<sup>24,25</sup>. Ludmer and colleagues<sup>23</sup> first showed that ACh infused into the coronary arteries causes normal epicardial dilation in patients with angiographically smooth coronary arteries, but dose-dependent abnormal constriction in patients with angiographic coronary artery disease (CAD). Clinically, several CAD risk factors identified with endothelial dysfunction are associated with decreased bioavailable NO, as evidenced by an abnormal coronary vasodilator response to ACh<sup>26</sup>. In patients at high risk for CAD, endothelial dysfunction is observed in morphologically intact vessels before the onset of clinically manifested vascular disease<sup>26</sup>. Failure of the endothelium to elicit NO-mediated vasodilation is caused by reduced bioavailability of endothelium-derived NO due to either decreased formation or accelerated degradation. Both excess generation of reactive oxygen species (ROS) including

superoxide anion and oxidized low density lipoprotein (LDL) cholesterol and decreased antioxidant defense mechanisms contribute to enhanced degradation of NO<sup>27</sup>. In experimental models of atherosclerosis and in human blood vessels, increased superoxide production is critically involved in reduced NO bioactivity and endothelial dysfunction<sup>28</sup>. In human blood vessels, increased superoxide production has also been associated with impaired NO-mediated vasorelaxation<sup>29</sup>.

Furthermore, it has been demonstrated that chronic oral administration of L-arginine to restore eNOS activity in hypercholesterolemic rabbits is associated with a reduction in the atherosclerotic lesion area and thickness<sup>30</sup>. Similarly, in atherosclerotic patients, intravenous L-arginine administration improves NO-mediated brachial artery reactivity<sup>31</sup>.

The role of endogenous NO in the progression of atherosclerosis in apolipoprotein E-deficient [apoE-knockout (KO)] mice was also studied<sup>32</sup>. Mice were treated with N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NOS or with L-arginine for 8 weeks. L-NAME treatment resulted in a significant inhibition of NO-mediated vascular responses and a significant increase in the atherosclerotic plaque/surface area in the aorta of apoE-KO mice. L-arginine treatment had no influence on endothelial function and did not alter lesion size. At the beginning of the study, impairment in endothelial function was only apparent in the case of N<sup>G</sup>-nitro-L-arginine-induced, NO-mediated contraction, whereas ACh-induced, NO-mediated relaxation was not different between age-matched apoE-KO and C57Bl/6J mice. After the 8-week treatment with the NOS inhibitor, both NO-mediated responses were significantly inhibited. The acceleration in lesion size concomitant with the severely impaired NO-mediated responses indicates that lack of endogenous NO is an important progression factor of atherosclerosis in the apoE-KO mouse.

Several studies in experimental models also support the involvement of NO-mediated pathways in vascular dysfunction and atherogenesis. NOS protein

is absent or reduced in the endothelium overlying severe atherosclerotic lesions in arteries<sup>33,34</sup>. It was found that ex vivo gene transfer of eNOS to atherosclerotic rabbit aortic rings improves relaxations to ACh<sup>35</sup>.

#### *Antioxidative effects of NO*

The oxidative stress hypothesis of atherosclerosis posits that it is an inflammatory disease triggered by subendothelial accumulation of LDL particles modified by ROS. Recent work, however, showed that ROS mediated many additional pathological processes in the vessel wall, including endothelial dysfunction as well as smooth muscle cell (SMC) migration, growth, and apoptosis<sup>36</sup>. In vitro studies demonstrated that proinflammatory stimuli activate proteins that generate both intracellular and extracellular ROS in virtually all vascular cells. ROS inactivate NO via three different mechanisms: direct inactivation of NO by superoxide, resulting in the formation of peroxynitrite<sup>37</sup>, reduced NOS activity due to increased levels of asymmetric dimethylarginine (ADMA), an endogenous NOS inhibitor<sup>38</sup>, and eNOS uncoupling due to increased oxidation of cofactor H<sub>4</sub>B<sup>39</sup>.

Among the various ROS formed under atherosclerotic conditions, superoxide is presumably the most important one. Increased vascular superoxide production has been demonstrated in all major conditions predisposing to atherosclerosis<sup>40</sup>. Furthermore, Sorescu et al.<sup>37</sup> have recently directly shown that there is increased superoxide production in coronary arteries in patients with coronary disease. Superoxide reacts rapidly with NO, thereby reducing NO bioactivity and producing peroxynitrite, a strong oxidant that can have physiologically protective effects but that at high levels causes tissue damage by nitrosylation of cellular proteins and lipids<sup>41</sup>. Furthermore, it has recently been demonstrated that increased vascular ROS production promotes the oxidative degradation of the critical eNOS cofactor H<sub>4</sub>B, leading to eNOS "uncoupling" with reduced NO production and increased superoxide production from the enzyme<sup>39,42</sup>.

Another antioxidative effect of NO is via the induction of extracellular superoxide dismutase (ecSOD), which has been shown to occur in vitro and in vivo (16). This potent antioxidative enzyme is expressed in vascular SMCs and located at the outer cell membrane. Fukai et al.<sup>43</sup> have put forward the concept that eNOS-derived NO induces ecSOD expression in the arterial wall as an important feed-forward mechanism to increase its biological effect; conversely, reduced NO availability would then reduce vascular ecSOD activity and further augment endothelial dysfunction. Indeed, recent observations in patients with coronary disease have supported this concept; in coronary arteries from patients with coronary disease, ecSOD activity was profoundly reduced<sup>44</sup>. In addition, superoxide stimulates mitogenesis in vascular SMCs and activates other redox-sensitive signaling pathways<sup>45,46</sup>. Increased superoxide production, principally by NADPH oxidases and xanthine oxidase, accounts for a significant proportion of the NO deficit in several models of vascular disease, including hypercholesterolemia<sup>47</sup> and atherosclerosis<sup>13,28</sup>. Correction of hypercholesterolemia reduces superoxide production and restores endothelium-dependent vasorelaxation<sup>48</sup>. Antioxidants<sup>49</sup> and SOD<sup>50</sup> produce similar effects, further supporting the hypothesis that superoxide plays a role in the observed NO deficits.

#### *Antiproliferative effects of NO*

Human atherosclerosis is a disease initially characterized by a focal intimal thickening of medium- and large-sized arteries. The migration and proliferation of vascular SMCs is a major component of atherosclerosis. The proliferating SMCs can migrate into the intima and contribute to intimal hyperplasia. Both proliferation and migration are controlled by various signaling molecules such as angiotensin II, TNF- $\alpha$  and several growth factors (transforming growth factor- $\beta$ , fibroblast growth factor, platelet-derived growth factor). NO is a potent inhibitor of SMC proliferation and migration in vitro, and that it may play a critical role in regulating vascular injury<sup>22,51,52</sup>. NO inhibits smooth muscle mitogenesis at distinct points in the cell cycle by cGMP-dependent

(late G1 phase)<sup>53,54</sup> and -independent (S phase)<sup>53,55</sup> mechanisms. In all animal models of vascular injury, the great majority of investigators have found that NO donors administered systemically or locally inhibit restenosis. NO donor molecules, of several structural classes, reduced intimal thickening in rabbits<sup>56, 57</sup>, pigs<sup>58</sup>, and rats<sup>59</sup>. Local administration of the NO precursor L-arginine also inhibited restenosis after balloon angioplasty in animal models<sup>60,61</sup>. In this situation, iNOS is responsible for NO production and L-arginine becomes rate-limiting<sup>62</sup>. There are intriguing data regarding the role of NO in restenosis after balloon angioplasty in patients with CAD. Recent data indicate that the ability of the vascular wall to produce NO after angioplasty correlates with less risk for restenosis<sup>63,64</sup>.

#### *Antiadhesive effects of NO*

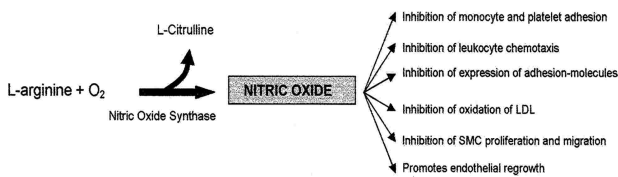
Recent advances have established a pivotal role for inflammation in all stages of atherosclerosis, including initiation, progression, and the complicated advanced lesion<sup>65</sup>. Increasing evidence suggests an important anti-inflammatory role of endothelium-derived NO. Injury to endothelial cell function, primarily resulting from increased oxidant stress within the endothelium<sup>28</sup>, leads to a cascade of events beginning with activation of vascular cytokines such as interleukin-1 and TNF- $\alpha$  and proceeding to expression of adhesion molecules on the cell surface that include vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and endothelial-leukocyte adhesion molecule (ELAM), which attracts monocytes and other leukocytes to adhere to the endothelial surface<sup>66,67</sup>. Adherence followed by infiltration of mononuclear cells into the vascular wall, together with activation of monocytes chemoattractant protein-1 (MCP-1), leads to scavenging of oxidized LDL, formation of lipid-laden foam cells, and development or progression of atherosclerotic plaque<sup>67</sup>. Lipids (particularly LDL) and oxidant stress play a major role in impairing endothelial function by reducing the bioavailability of NO and activating pro-inflammatory signaling pathways such as nuclear factor kappa B (NF $\kappa$ B).

NF $\kappa$ B is a redox-sensitive transcription factor which regulates, in part, gene expression of many cytokines, growth factors, adhesion molecules and enzymes involved in immune and inflammatory responses<sup>68</sup>. NO donors inhibit activation and nuclear translocation of NF $\kappa$ B<sup>69,70</sup>, block cytokine-stimulated endothelial adhesion molecule expression<sup>70</sup>, and reduce adhesion and activation of neutrophils and monocytes<sup>71</sup>. Pharmacologic inhibition of endothelium-derived NO production leads to a marked increase in the endothelial adhesiveness for monocytes, and this effect is attenuated by dietary L-arginine, the substrate of eNOS. Similarly, increased leukocyte endothelial cell interactions have been observed in eNOS-deficient mice<sup>72</sup>.

#### **NITRIC OXIDE AND ATHEROSCLEROSIS**

Atherosclerosis is a multifactorial disorder and the main cause of morbidity and mortality in many countries, and many forms of cardiovascular disease are associated with this disorder. Atherosclerosis is described as a disease of large- and medium-sized muscular arteries and is characterized by endothelial dysfunction, vascular inflammation, and buildup of lipids, cholesterol, calcium, and cellular debris within intima of the vessel wall. This buildup results in plaque formation, vascular remodeling, acute and chronic luminal obstruction, abnormalities of blood flow, and diminished oxygen supply to target organs. Atherosclerotic blood vessels exhibit several functional changes such as enhanced vasoconstriction, increased adhesion of platelets and monocytes as well as migration and proliferation of vascular SMCs. Under all conditions, an impaired NO production could contribute because this molecule plays a role in all these processes (Figure 1)<sup>73-76</sup>. Atherosclerosis is associated with reduced endothelial NO production, and in patients with CAD, even angiographically normal coronary segments show paradoxical constriction to ACh<sup>23</sup>. Endothelial function is also deficient in preatherosclerotic conditions such as hypercholesterolemia<sup>77,78</sup>, hypertension<sup>79</sup>, and normal aging<sup>77</sup>. The observation of deficient NO-mediated vasorelaxation in hypercholesterolemia suggests that loss of NO bioactivity is an early feature of

atherosclerosis<sup>80</sup>. Several mechanisms have been proposed to explain the impaired endothelium-dependent relaxations in atherosclerosis, including alterations in endothelial signal transduction, reduced availability of L-arginine, modification of eNOS and co-factors for eNOS, inhibition of NO by ROS, reduced response of vascular smooth muscle to NO, and intimal thickening formation as a diffusion barrier (81).



**Figure 1.** Summary of vasoprotective effects of nitric oxide in atherosclerosis.

After the initial endothelial dysfunction and reduced NO bioavailability, a pro-inflammatory phenotype is seen in animals predisposed to atheromatous plaque formation, with neutrophil adherence<sup>13</sup>. Endothelium-derived NO is known to be an anti-inflammatory and anti-atherosclerotic molecule; mice lacking the endothelial-type NO synthase gene exhibit hypertension and enhanced vascular remodeling in response to injury<sup>82,83</sup>. An induction of the generation of ROS leads to cellular injury and peroxidation of lipid components. This produces oxidized LDLs, which are the key mediators of atherosclerosis. Oxidized LDL may reduce eNOS levels by inhibiting eNOS gene expression<sup>84</sup> and also can displace eNOS from its position in the intracellular caveolae and therefore disrupt eNOS activity<sup>13</sup>. NOS gene therapy aims to increase or restore vascular NO production in these deficiency states. The transfer of the NO synthase gene improves vascular reactivity<sup>85,86</sup> and reduces atherogenesis in animals<sup>87</sup>.

## THERAPEUTIC INTERVENTIONS

Atherosclerosis develops as a result of a healing response to repetitive injury to the arterial wall. Numerous risk factors contribute to the development and progression of atherosclerosis and of these, endothelium-derived NO is the key factor protecting the artery, which in turn prevents the ischemic

manifestations of atherosclerosis. Therefore, endothelium-derived NO is a clinically relevant therapeutic target for cardiovascular diseases. Current research is examining strategies that might improve arterial endothelial function. A number of lifestyle changes, nutritional interventions and pharmacotherapies have been shown to improve endothelial vasodilator function; several of these therapies have also been shown to improve perfusion and function of target organs, and to reduce cardiovascular events.

Regular exercise is an important lifestyle factor that reduces cardiovascular risk<sup>88</sup>. In addition, regular exercise directly increases NOS expression and activity in the vessel<sup>89</sup>. In contrast, a sedentary lifestyle is associated with endothelial dysfunction, increased oxidative stress, and elevated systemic markers of inflammation. Diets low in fat and high in fruits and vegetables improved endothelial vasodilator functions<sup>90</sup>. Endothelial dysfunction is reversed after intake of flavonoid-containing beverages including tea<sup>91</sup>, grape juice<sup>92</sup>, and red wine<sup>93</sup>. In patients with vascular disease, fish oil, phytoestrogens or L-arginine have been shown to improve endothelium-dependent vasodilation<sup>94-96</sup>.

Oxidative stress is a central cause of endothelial dysfunction in atherosclerosis, and there has been great interest in the effects of antioxidant therapy. Several studies have focused on the use of antioxidants to improve endothelial dysfunction in animals<sup>97,98</sup> and humans<sup>99-101</sup>. For example, it has been shown that the oxygen radical scavengers vitamin C and probucol improved impaired endothelium-dependent vasodilation in hypercholesterolemia and atherosclerosis<sup>100</sup>.

Some pharmacological agents are also known to improve endothelial dysfunction in atherosclerosis, including angiotensin-converting enzyme inhibitors<sup>102,103</sup> and lipid-lowering drugs<sup>100,104</sup>. Inhibition of angiotensin II reduces superoxide generation from blood vessels and may increase bioavailability of NO, and bradykinin potently stimulates endothelial cells to release NO<sup>105</sup>. Statins

increase the expression of NOS in the vessel wall, whereas angiotensin converting enzyme inhibitors reduce the breakdown of bradykinin, and thereby increase the stimulation of NOS activity<sup>106</sup>. Statin therapy has also been shown to modulate vascular tone by decreasing the synthesis of the NO antagonist endothelin-1<sup>107</sup>.

Gene therapy for vascular disease will be the next clinical challenge. The advantages of gene replacement therapy have allowed for local delivery of adenoviral vectors to express eNOS and restore NO production to the vessel wall following injury<sup>108</sup>. Sinnaeve et al.<sup>109</sup> showed that local adenovirus-mediated gene transfer of guanylyl cyclase  $\alpha 1$  and  $\alpha 1$  subunits in a rat carotid injury model resulted in a 60-fold increase in NO-stimulated cGMP levels, which was associated with a significant decrease in cell proliferation and migration. NO replacement therapies offer therapeutic benefit in the setting of the endothelial dysfunction, and may correct the impaired vascular responses associated with depletion of NO stores. □

In summary, it is clear that the vascular endothelium importantly influences the physiology of blood vessels and appears to be central in mediating damage to the vessel wall when patients are exposed to conventional risk factors for atherosclerosis. It is likely that future therapy will be targeted towards improving vascular endothelial function by utilizing more than one of the strategies outlined above to achieve a long-term impact on atherosclerosis and its adverse manifestations.

## CONCLUSION

Endothelium and its major product NO are key regulators of vascular function. Production of NO is crucial for maintenance of normal vascular endothelial integrity. Some of the most important effects that NO exerts in the vascular wall are potentially vasoprotective, because these effects maintain important physiological functions such as vasodilation, anticoagulation, leukocyte adhesion, smooth muscle proliferation, and the antioxidative capacity. Impaired bioavailability of NO is involved

in the initiation, progression and complications of atherosclerosis. Therefore, therapeutic approaches in the prevention and the treatment of atherosclerosis based on NO bioactivity can become a challenge for future studies.

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