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# CLINICIAN UPDATE

## Fundamentals of Endothelial Function for the Clinical Cardiologist

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**C**ase presentation 1: Mr Gundry is a 42-year-old construction worker who has had type II diabetes mellitus for the past 6 years. He has no other vascular risk factors and is currently asymptomatic. Is this patient at risk of developing endothelial dysfunction?

Case presentation 2: Mr Sinha is a 64-year-old man who presents to your office for symptomatic intermittent claudication. Past medical history is unremarkable except for the presence of multiple vascular risk factors (smoking, hypertension, dyslipidemia, and obesity). How do vascular risk factors cause endothelial dysfunction? Is endothelial dysfunction treatable?

A survey of medical history will reveal that major breakthroughs are often a result of simple and at times unexpected observations. Indeed, the era of endothelial biology was brought to the forefront through a simple pharmacological experiment conducted approximately 2 decades ago in Dr Furchgott's laboratory.<sup>1</sup> In this landmark study, the authors described the essential role of the endothelium in mediating the vasodilatory actions of acetylcholine, now known to be dependent on the release of nitric oxide (NO) from the endothelium. No one could have predicted that this observation would have the gargantuan impact on vascular biology that is evident today. Endothelial dysfunction now is implicated in the pathogenesis and clinical course of the majority of cardiovascular diseases. The purpose of this "Clinical Cardiology: Physician Update" is to provide the busy cardiovascular specialist with some of the fundamental principles of endothelial function as they relate to cardiovascular health and disease.

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### How Does the Endothelium Maintain Vascular Homeostasis?

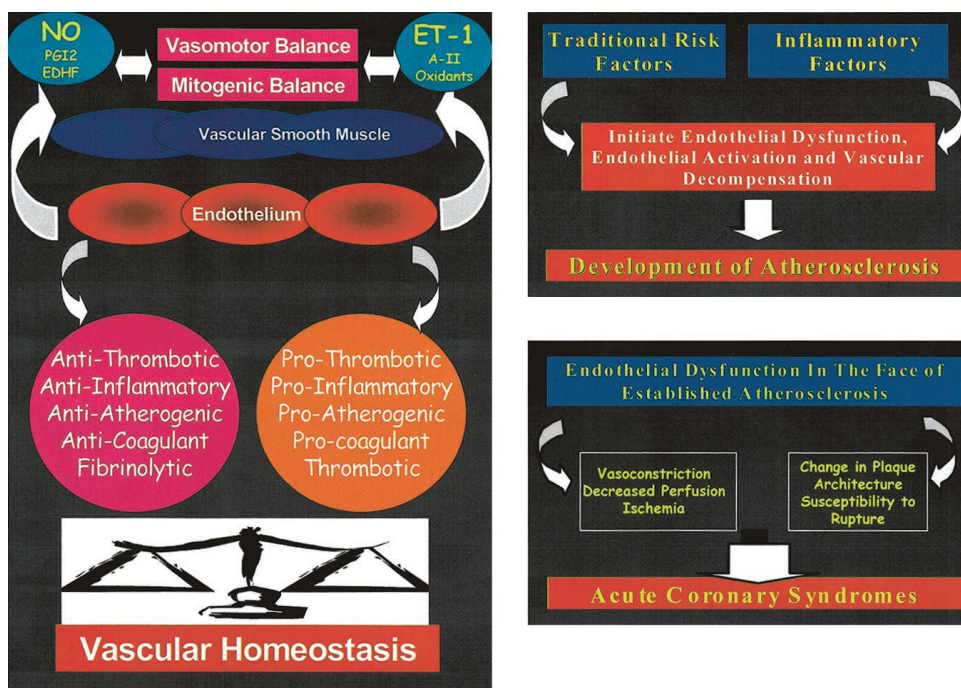
The endothelium is a single-cell lining covering the internal surface of blood vessels, cardiac valves, and numerous body cavities. The strategic location of the endothelium allows it to "sense" changes in hemodynamic forces and blood-borne signals and "respond" by releasing vasoactive substances. A critical balance between endothelium-derived relaxing and contracting factors maintains vascular homeostasis. When this balance is disrupted, it predisposes the vasculature to vasoconstriction, leukocyte adherence, platelet activation, mitogenesis, pro-oxidation, thrombosis, impaired coagulation, vascular inflammation, and atherosclerosis (Figure).

NO is the key endothelium-derived relaxing factor that plays a pivotal role in the maintenance of vascular tone and reactivity.<sup>2</sup> In addition to being the main determinant of basal vascular smooth muscle tone, NO opposes the actions of potent endothelium-derived contracting factors such as angiotensin-II and endothelin-1 (ET-1) (Table 1). NO inhibits platelet and leukocyte activation and maintains the vascular smooth muscle in a nonproliferative state. NO is synthesized from L-arginine under the influence of the enzyme nitric oxide synthase (NOS). NOS requires a critical cofactor, tetrahydrobiopterin, to facilitate NO production.<sup>3</sup> Tetrahydrobiopterin deficiency leads to an "uncoupling of NOS" with the resultant production of potent oxidants such as superoxide and hydrogen peroxide.

In addition to its production of angiotensin-II, the endothelium is the source of the potent vasoconstrictor peptide ET-1.<sup>4</sup> ET-1 augments the vascular actions of other vasoactive peptides such as angiotensin-II, norepinephrine, and serotonin; participates actively in leukocyte and platelet activation; and facilitates a prothrombotic and proatherogenic phenotype.

### What Role Does Endothelial Dysfunction Play in Cardiovascular Pathophysiology?

Although studies often report endothelial dysfunction as a loss of the vasodilatory capacity (in response to a NO



Left, The role of the endothelium in the maintenance of vascular homeostasis. Endothelium-derived vasoactive factors come into concert to maintain vasomotion, smooth muscle proliferation, thrombosis, inflammation, coagulation, fibrinolysis, and oxidation. Endothelial dysfunction is defined as the loss of one or more of these functions with resultant vascular decompensation. Top right, Traditional and nontraditional risk factors lead to endothelial dysfunction. Endothelial dysfunction plays an integral role in the initiation of atherosclerosis. Bottom right, Endothelial dysfunction assumes extra importance in the face of existing atherosclerosis. Atherosclerotic coronary arteries respond to endothelium-dependent stimuli by evoking paradoxical vasoconstriction. Furthermore, endothelial dysfunction promotes plaque instability and rupture. These events may lead to the development of unstable coronary syndromes.

stimulus, like acetylcholine), the term encompasses a generalized defect in all the homeostatic mechanisms. Endothelial dysfunction is a broad term that implies diminished production of or availability of NO and/or an imbalance in the relative contribution of endothelium-derived relaxing and contracting factors (such as ET-1, angiotensin, and oxidants). For example, endothelial dysfunction in diabetes may result from a decreased bioavailability of NO (secondary to insulin resistance) coupled with an exaggerated production of ET-1 (stimulated by hyperinsulinemia or hyperglycemia).<sup>5</sup> Endothelial dysfunction has been implicated in the pathogenesis and clinical course of all known cardiovascular diseases and is associated with future risk of adverse cardiovascular events.<sup>6</sup> (Table 2). We focus our discussion on the role of endothelial dysfunction in the development and progression of atherosclerosis.

It is well accepted that endothelial dysfunction occurs in response to cardiovascular risk factors and precedes the development of atherosclerosis.<sup>7,8</sup> The balance of published information supports the paradigm of endothelial dysfunction as the common link between risk factors and atherosclerotic burden. Endothelial dysfunction actively participates in the process of lesion formation by promoting the early and late mechanisms of atherosclerosis. These include upregulation of adhesion molecules, increased chemokine secretion and leukocyte adherence, increased cell permeability, enhanced LDL oxidation, platelet activation, cytokine elaboration, and vascular smooth muscle cell proliferation and migration.

Endothelial dysfunction also plays an important role in the clinical course of atherosclerosis. Impaired endothelium-dependent vasodilatation in coronary arteries with established atherosclerosis results in paradoxical vasoconstriction, which

**TABLE 1. Autocrine and Paracrine Substances Released From the Endothelium**

Vasodilators	NO, prostacyclin, endothelium-derived hyperpolarizing factor, bradykinin, adrenomedullin, C-natriuretic peptide
Vasoconstrictors	ET-1, angiotensin-II, thromboxane A <sub>2</sub> , oxidant radicals, prostaglandin H <sub>2</sub>
Antiproliferative	NO, prostacyclin, transforming growth factor-β, heparin sulphate
Proproliferative	ET-1, angiotensin-II, oxidant radicals, platelet-derived growth factor, basic fibroblast growth factor, insulin-like growth factor, interleukins
Antithrombotic	NO, prostacyclin, plasminogen activator, protein C, tissue factor inhibitor, von Willebrand factor
Prothrombotic	ET-1, oxidant radicals, plasminogen-activator inhibitor-1, thromboxane A <sub>2</sub> , fibrinogen, tissue factor
Inflammatory markers	CAMs (P- and E-selectin, ICAM, VCAM) chemokines, nuclear factor κ-B
Permeability	Receptor for advanced glycosylation end-products
Angiogenesis	Vascular endothelial growth factor

**TABLE 2. Conditions Associated With Impaired Endothelial Function, Strategies to Counter Endothelial Dysfunction, and Surrogate Soluble Markers of Endothelial Dysfunction**

Conditions associated with impaired endothelial function	Atherosclerosis, hypercholesterolemia, high LDL-C, low HDL-C, high lipoprotein (a), small dense LDL-C, oxidized LDL-C, hypertension, high homocysteine, aging, vasculitis, preeclampsia, metabolic syndrome, variant angina, diabetes, active smoking, passive smoking, ischemia-reperfusion, transplant atherosclerosis, cardiopulmonary bypass, postmenopausal, Kawasaki's disease, Chagas' disease, family history CAD, infections, depression, inactivity, obesity, renal failure, increased CRP, congestive heart failure, left ventricular hypertrophy, postprandial state
Interventions to improve endothelial function	ACE inhibitors, angiotensin receptor blockers, endothelin blockers, statins, tetrahydrobiopterin, folates, exercise, improving insulin sensitivity, LDL reduction, HDL augmentation, antioxidants, estrogen, L-arginine, desferoxamine, glutathione, homocysteine reduction, lowering CRP, reducing free fatty acid flux
Soluble surrogate markers of endothelial dysfunction	CAMs, von Willbrand factor, ET-1, nitrites, asymmetric dimethylarginine, CRP, tissue plasminogen activator, fibrinogen, amyloid A

may result in reduced myocardial perfusion and myocardial ischemia. Additionally, endothelial dysfunction actively modulates plaque architecture and portends the vulnerability of the lesion and the likelihood of rupture. Through this vasoconstrictor and inflammatory mechanism, endothelial dysfunction in atherosclerotic vessels may lead to the development of unstable coronary syndromes (Figure).<sup>9</sup>

### Is Endothelial Dysfunction Treatable?

Interventions that restore endothelial function have important clinical implications (Table 2). We briefly discuss the role of cholesterol reduction and angiotensin antagonism as strategies to improve endothelial health.

There is overwhelming evidence to support the notion that LDL cholesterol reduction augments endothelial-dependent vasodilatation.<sup>10</sup> Although a number of modalities that lower cholesterol have been demonstrated to improve endothelial function, much recent attention has focused on the statin class of antihyperlipidemic agents. There now is convincing evidence that statins exert direct, cholesterol-independent (pleiotropic) effects to improve endothelial function.<sup>11</sup> Such actions may underlie the early and profound effects of these drugs on cardiovascular morbidity and mortality.

The other class of drugs with dramatic effects on endothelial function consists of angiotensin-converting enzyme inhibitors (ACEIs). Like statins, the beneficial effects of ACEIs have been demonstrated in the coronary and peripheral circulations; importantly, these effects become apparent after short-term treatment.<sup>12,13</sup> ACEIs improve endothelial function through a variety of mechanisms: They have antioxidant properties, and they can produce a favorable effect on fibrinolysis, a decrease in angiotensin-II, and an increase in bradykinin. Although a causal relationship between ACEI-induced improvements in endothelial function and reduction in cardiovascular events has not been clearly established, the wealth of available evidence suggests that improved endothelial function might be one of the important underlying mechanisms.

### How Is Endothelial Function Assessed?

Endothelium-dependent vasodilatation can be assessed in the coronary and peripheral circulations. Here we provide a snapshot of the available modalities of testing and refer the reader to comprehensive reviews on the topic.<sup>14,15</sup>

### Coronary Circulation

Quantitative coronary angiography can be used to examine the change in diameter in response to intracoronary infusions of endothelium-dependent vasodilators such as acetylcholine. In healthy vessels, acetylcholine evokes a NO-mediated vasodilatory response; in patients with endothelial dysfunction, this effect is blunted or vasoconstriction is paradoxical. Endothelial function of the coronary microvasculature can be assessed with intracoronary Doppler techniques to measure coronary blood flow in response to pharmacological or physiological stimuli. Noninvasive tests for assessment of coronary endothelial function include Doppler echocardiography, positron emission tomography, and phase-contrast magnetic resonance imaging.

### Peripheral Circulation

Brachial artery ultrasound is a widely used noninvasive measure of endothelial function. Upper-arm occlusion for 5 minutes results in reactive hyperemia after the cuff is released; this increase in shear stress results in endothelium-dependent flow-mediated vasodilatation. Importantly, endothelial dysfunction assessed by this technique correlates with measures of coronary endothelial dysfunction.<sup>16</sup> Peripheral resistance vessel function can be assessed by strain-gauge venous impedance plethysmography.<sup>17</sup> This technique examines the change in forearm blood flow in response to direct intra-arterial (brachial artery) administration of agonists. Noninvasive measures of arterial compliance and waveform morphology provide a marker of vascular health.

### How Do Inflammatory Markers of Atherosclerosis Relate to Endothelial Dysfunction?

A number of circulating markers of endothelial dysfunction and vascular inflammation have been studied over the past few years (Table 2). We limit our discussion to soluble cellular adhesion molecules (CAMs) and C-reactive protein (CRP).

CAMs are expressed on the surface of endothelial cells and leukocytes in response to endothelial dysfunction. The 3 major classes of CAMs include (1) the selectins (P-selectin, L-selectin, E-selectin), (2) the  $\beta_2$  integrins (CD11/CD18), and (3) immunoglobins (intercellular adhesion molecule-1 [ICAM-1], vascular cell adhesion molecule-1 [VCAM-1], and platelet endothelial cell adhesion molecule-1 [PECAM-

1]). CAMs orchestrate the complicated process of leukocyte rolling, adhesion, and transmigration into the subintimal space. Circulating levels of CAMs have been examined as surrogate markers of endothelial function. Elevated levels of CAMs have been observed in patients with cardiovascular risk factors and may predict the development of cardiovascular disease.

High-sensitivity CRP has been studied widely as a predictor of cardiovascular disease.<sup>18</sup> Accumulating evidence suggests that atherosclerosis represents a chronic inflammatory process, and hence, inflammatory markers like CRP may provide an adjunctive method for global assessment of cardiovascular risk. Several large-scale studies have shown that plasma levels of CRP are a strong independent predictor of endothelial dysfunction, future myocardial dysfunction, stroke, peripheral artery disease, and vascular death among individuals without known cardiovascular disease.<sup>19</sup> Recent data suggest that CRP may directly promote endothelial dysfunction by increasing the synthesis of CAMs, increasing monocyte chemoattractant protein (MCP)-1 secretion, and facilitating macrophage LDL uptake.<sup>20</sup>

### What Does the Future Hold?

The future holds great promise. We will witness an increasing number of therapeutic strategies aimed at improving endothelial function in a variety of cardiovascular disease states. The next decade will witness an exponential interest in developing reliable methods of testing endothelial function as a potential predictor of cardiovascular disease. Several large noninvasive studies currently are underway to determine the predictive value of brachial ultrasound testing. Inflammatory markers, such as CRP, likely will find their way into risk assessment algorithms. As measures of endothelial dysfunction become clinically applicable, this may translate into improved methods of risk assessment and equip physicians with yet another tool to predict, prevent, and treat cardiovascular disease. Amid the excitement and the thrill that is shared by vascular biologists worldwide today, we remain indebted to the simple pharmacological experiment that ushered in the era approximately 2 decades ago.

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