A growing body of evidence suggests that endothelial dysfunction is associated with cardiovascular events. Endothelial dysfunction can be regarded as a syndrome that exhibits systemic manifestations associated with significant morbidity and mortality. The concept of endothelial dysfunction should be extended beyond the conduit vessels into the vascular wall and even to the bone marrow and the progenitor endothelial cells. The present review is focused on the potential underlying mechanism by which endothelial dysfunction may contribute to cardiovascular events. The assessment of endothelial function may emerge as an integral adjunct test for evaluation of the vulnerable patients at risk for future cardiovascular events.

Background
Atherosclerosis is a chronic, systemic, and diffuse disease with focal complications in different vascular beds. The mechanisms by which a specific site is rendered more prone to the development of symptomatic disease and cardiovascular events are not known. The observation that all stages of atherosclerosis may be at distant and at multiple locations simultaneously but at the same time may spare entire segments may give rise to the hypothesis that the interface and interaction between the vascular wall and the circulation is the primary site of the mechanism underlying cardiovascular events.1

The endothelium is the monolayer of endothelial cells lining the lumen of the vascular beds and is mechanically and metabolically strategically located, separating the vascular wall from the circulation and the blood components.1,2 To fully understand the mechanism by which alteration in endothelial function may lead to cardiovascular events, we need to extend our vision and concepts regarding the nature and function of the endothelium.

Localization and Distribution of the Endothelium
The first concept that needs to be revised is the localization and distribution of the endothelial cell layer. The focus in the past decade has been on the endothelial layer of the large conduit vessels and the vascular lumen.2 However, the vascular bed extends into the vascular wall and the adventitial vasa vasorum, which is also considered an active intravascular microcirculation and is also lined by endothelium. Thus, the concept of endothelial function should be extended to the vascular wall itself and the adventitia.2

An additional novel view of the endothelium may be the consideration of the origin of the endothelial cells and inclusion of the bone marrow and the endothelial progenitor cells (EPCs). The vascular wall and the endothelium in particular are undergoing a constant process of injury and repair in response to mechanical and chemical injuries. Emerging evidence suggests that bone marrow–derived endothelial stem cells and EPCs contribute to the repair of vascular injury and play a role in restoration of tissue repair.4 The bone marrow contains vascular progenitor cells that can mobilize to the injury site and complement repair afforded by preexisting endothelium. Despite experimental evidence demonstrating the contribution of bone marrow–derived progenitor cells to tissue revascularization, the importance of these cells in repairing vascular damage in the clinical setting remains unknown. At the site of tissue vascularization, endothelial cells can originate either from adjacent preexisting blood vessels or from recruitment of bone marrow–derived circulating EPCs. Interestingly, the repair of vascular injury with EPCs is associated with normalization of endothelial function at the site of the injury.5 Conversely, the repair of vascular injury may be impaired in the setting of endothelial dysfunction, which may be attributed to 2 possible mechanisms. A recent study demonstrated that the degree of endothelial dysfunction correlated with the number of EPCs. Thus, one of the possible mechanisms for vascular endothelial dysfunction may be a relative deficiency of EPCs for vascular repair.6 Moreover, the function of these cells and their ability to participate in the vascular repair after injury has been shown to be impaired in an animal model of decreased nitric oxide (NO) activity.7 It may be speculated that endothelial NO activity, the hallmark of endothelial function, may be reduced at the level of the multipotential cells of subjects with systemic endothelial dysfunction and atherosclerosis. This notion is further supported by the observations that in type II diabetes, a condition that is associated with endothelial dysfunction, recruitment of EPCs to the site of tissue repair is diminished,8 and that statins, one of the major pharmacological interventions that is known to improve endothelial function, promotes the mobilization and
the function of EPCs.9,10 Thus, the concept of endothelial dysfunction as a syndrome with various clinical presentations rather than a localized vascular disorder may explain the mechanism of cardiovascular events that are associated with endothelial dysfunction.

Clinical Implications of Endothelial Dysfunction

The clinical implications of endothelial dysfunction and the association between endothelial cell dysfunction and cardiac events are well established and reviewed.11,12 A multivariant analysis of the present studies describing the association between coronary or peripheral endothelial dysfunction and cardiovascular events is illustrated in Figure 1. In this analysis, close to 2500 patients were included, with a duration of follow-up between 1 and 92 months. The nature of the cardiovascular events varied among the studies but overall represents major cardiovascular events such as cardiac death, myocardial infarction, and need for revascularization. This analysis demonstrates that endothelial dysfunction is strongly and independently associated with cardiovascular events. The similar power of coronary and peripheral endothelial dysfunction to predict cardiovascular events and the observation that the cardiovascular events may occur remotely from the site in which the endothelial dysfunction was detected13 underscore the systemic nature of endothelial dysfunction and its pivotal role in prediction of cardiovascular events. Moreover, the lack of direct correlation between the presence of endothelial dysfunction and traditional risk factors further supports the concept that endothelial dysfunction may be regarded as the integrated risk of the risk factors and serves as a sensitive marker for their functional significance.1 Development and introduction of novel, cost-effective, noninvasive, and reproducible tests to evaluate endothelial function may conceivably promote the use of these tests for clinical application and practice.

Assessment of Endothelial Function

Endothelial function may be tested by 2 main methods. The primary method provides more direct information on the functional capacity of the endothelium and involves evaluation of the endothelial cell response to direct stimulation of the endothelium and may be considered as endothelial stress tests. These tests are based on 2 main principles: the first is that certain stimuli trigger the release of NO from the vascular endothelium to mediate vascular relaxation. The second principle is that endothelial dysfunction is a systemic disorder and thus can be measured in different vascular beds (Figure 2). Endothelial vasomotor testing may be performed in the coronary circulation by use of state-of-the-art coronary angiography and intracoronary Doppler for the direct calculation of changes in coronary blood flow and coronary vascular resistance.14,15 Although this method of functional angiogram is considered to be the “gold standard” for the assessment of endothelial function, its invasive nature and the need for special expertise and equipment hamper its use. The alternative endothelial vasomotor test involves the peripheral circulation. Endothelial function of forearm resistance vessels can be assessed by intra-arterial infusion of endothelium-dependent vasodilators similar to those used in the coronary circulation, such as acetylcholine.12 A less invasive test that gained popularity primarily in the research arena is the assessment of endothelium-dependent flow-mediated dilation of the brachial artery by use of high-resolution ultrasound. Recent reviews summarized the benefits and the limitation of each of these methods, underscoring the need for develop-
ment of better noninvasive approaches to test endothelial function. The other indirect test that is used to gain information on the status of the endothelium is the measurement of peripheral markers that are associated with endothelial dysfunction and the progression of inflammation and atherosclerosis. The vascular activation of endothelial cells represents a critical step in atherosclerosis. The assessment of circulating markers of vascular wall inflammation represents a promising tool for predicting cardiovascular risk and outcome after cardiovascular events. Other factors that are released by the endothelium, such as endothelin-1 and NO, may also be used to assess the activation of the endothelium. The use of these markers is based on the hypothesis that endothelial dysfunction is characterized by an imbalance between endothelium-derived vasodilators that have anti-inflammatory and antimitogenic properties and vasoconstrictors with proatherogenic activity.

In the following segments, we will review the possible mechanism by which endothelial dysfunction may contribute to specific cardiovascular events.

Acute Coronary Syndrome and Sudden Death

Recent observations have demonstrated that acute coronary syndrome (ACS) and sudden cardiac death (SCD) cannot be predicted by nor are necessarily associated with significant obstructive coronary artery disease. The importance of understanding the potential role of endothelial dysfunction in SCD is underscored by the observations that SCD is the first and terminal manifestation of coronary artery disease in more than 50% of all cases and that more than two thirds of the victims had no documented coronary artery disease before death. These events are likely to be dependent on the vascular wall properties and components of the plaque, its vulnerability, and its interaction with the circulation. The endothelium regulates all these activities in the plaque as well as the thrombogenicity and the vulnerability of the circulation.

There are several phases at which the endothelium may play a role in the pathogenesis of ACS.

Plaque Rupture or Erosion

Plaque destabilization, the detrimental process that predisposes the plaque to induce cardiac events, results from a complex interplay of inflammatory effects that involve cellular plaque components and various proinflammatory mediators (cytokines, lymphokines, chemokines). Endothelial dysfunction is associated with increased oxidative stress, an important promoter of inflammatory processes. The process of inflammation is regulated in part by NO, which may reduce endothelial expression of several inflammatory mediators and adhesion molecules that increase plaque vulnerability. This effect is mediated primarily by inhibition of the transcription factor nuclear factor-kB, a key regulator of various inflammatory proteins involved in atherosclerosis. Thus, a dysfunctional endothelium may contribute to plaque destabilization and plaque erosion because of its reduced antiinflammatory and antioxidative potential.

Vasoconstriction

Precipitation of ACS may also involve physical factors related to endothelial dysfunction. Given the increased vaso-reactivity favoring local vasoconstriction in response to metabolic and sympathetic stimuli found in the area of culprit lesions in patients with unstable angina, vasoconstriction associated with endothelial dysfunction may obviously represent a trigger for the physical disruption of coronary plaques. Moreover, the release of the potent vasoconstrictor endothelin-1, which is characteristic of endothelial dysfunction, is enhanced at the site of the unstable plaque, further mediating focal vasoconstriction and myocardial ischemia. The enhanced vasoconstriction at the site of the active plaque may escalate further in response to such stimuli as physical and mental stress, which mediate vasoconstriction via an endothelium-dependent mechanism.

Vascular Vasa Vasorum Endothelial Function and Hemorrhage

As mentioned earlier, endothelial dysfunction may also affect the vascular microcirculation, which includes the vasa vasorum. The vasa vasorum surround and penetrate the adventitia and outer media of large arteries and veins in most vascular beds, including the coronary, renal, carotid, femoral, and pulmonary arteries. These microvessels consist of layers of smooth muscle oriented radially around a single layer of endothelium, indicating that they have the capacity to regulate their own tone independently of the host vessel and may therefore also develop endothelial dysfunction independently of the host vessel. Moreover, transfection of endothelial NO synthase to the vascular adventitia restores endothelium-dependent relaxation in the host vessel, even after endothelial denudation, underscoring the functional significance of the adventitial vasa vasorum in regulating vasomotion.

The impact of endothelial dysfunction in the vasa vasorum is likely to be substantial, because their vascular area is more than 70 times that of the same length of the mother vessel. Thus, systemic exposure of the endothelial cell surface to similar risk factors may lead to endothelial dysfunction of the vascular bed in the vascular wall, in turn leading to vascular wall ischemia and to neovascularization. Intraplaque neovascularization may lead to further enhanced influx of macrophages into the plaque as well as to intraplaque hemorrhage, contributing to ACS and cardiac death.

In the first stages of atherosclerotic plaque development, cells in the plaque receive oxygen by diffusion from the arterial lumen. Experimental and human pathology studies have shown that the number of both adventitial vasa vasorum and of intraplaque capillaries increases with plaque progression. When intimal thickness increases beyond the critical diffusion limits, vasa vasorum and intraplaque neovessels appear to oxygenate the plaque, a process that is regulated by the endothelium. However, parts of advanced plaques become sensitive to local vasoconstrictors and are rendered hypoxic. In advanced plaques, hypoxia-inducible factor-1-alpha as well as vascular endothelial growth factor are upregulated, suggesting the presence of hypoxia and activation of angiogenesis pathways. The resultant fragile neovessels can rupture or leak and cause intraplaque hemorrhage. Consequently, plaques will grow expansively, plaque hypoxia and neovascularization will redevelop, and new intraplaque hemorrhages will occur. This cycle
might eventually lead to total occlusion of the arterial lumen in association with thrombus formation.

Endothelial Function and the Vulnerable Plaque

Vulnerable patients often present with multiple plaques with erosion and undergoing rupture. In an angiography study of patients with an ACS, 39.5% of the patients had multiple complex plaques that were associated with an increased incidence of recurrent ACS. In another study with intravascular ultrasound, 79% of the patients presenting with an ACS had multiple ruptured plaques at sites other than the culprit lesion that caused the clinical symptoms. It can therefore be postulated that local endothelial dysfunction, in association with inflammation, enhanced platelet aggregation, coagulopathy, and the occluding thrombus at the culprit lesion determines the clinical presentation. However, it is only a focal manifestation of an underlying systemic disease process that includes several rupture-prone or vulnerable lesions.

Endothelial Function and the No-Reflow Phenomenon

Endothelial dysfunction may also play a role in the no-reflow phenomenon after restoration of coronary blood flow or after coronary intervention. Microscopic examination of the territory of the infarct-related artery reveals extensive endothelial damage at the level of the coronary microcirculation. Moreover, the release of endothelium-derived vasoconstrictor factors such as endothelin-1 from the site of coronary intervention may potentially contribute to the reduced flow and the extension of myocardial ischemia.

Several studies over the past few years have described the phenomenon of an acute and persistent endothelial dysfunction after exposure to a variety of stimuli such as endotoxin or reperfusion. This phenomenon was called “endothelial stunning” and may lead to myocardial ischemia. A recent study demonstrated that this phenomenon may also contribute to the extension of myocardial ischemia after acute changes in coronary blood flow. Endothelial stunning may be secondary to an acute increase in oxidative stress and may be reversed by the supplementation of L-arginine or angiotensin antagonist. This concept is further supported by the observations that pretreatment with endothelium-protecting agents such as statins is associated with reduced myocardial injury after coronary intervention and ACS.

Endothelial Response to Injury and Platelet Adhesion

A healthy endothelium sustains an antithrombotic milieu by secretion of various factors that exert antiaggregatory effects on platelets (NO and prostaglandin I2) or have anticoagulatory (heparin and protein C/S) or fibrinolytic (tissue plasminogen activator) properties. In contrast, endothelial dysfunction is characterized by a reduction in NO, prostacyclin, and tissue plasminogen activator, thereby contributing to vasoconstriction mediated by endothelin, serotonin, and thrombin. A decrease in the anticoagulatory potential of the endothelium and an increase in endothelial production of procoagulatory mediators (eg, tissue factor and plasminogen activator inhibitor) result in a thrombogenic vascular environment that allows thrombus formation induced by exposure of highly thrombogenic substances from ruptured or erosive plaques. In addition, platelet-derived mediators, such as serotonin, induce vasoconstriction in the presence of a dysfunctional endothelium.

One linkage between thrombosis and inflammation can be seen at the molecular and cellular levels in the endothelium. The primary molecules responsible for initiation of platelet and leukocyte adhesion, von Willebrand factor and P-selectin, respectively, are stored in the same storage granules, called Weibel-Palade body formation. As a result of endothelial and cellular activation, Weibel-Palade bodies fuse with the cell membrane and expose membrane-bound P-selectin and soluble von Willebrand factor at the cell surface. This process further propagates the inflammatory process and may promote platelet activation and aggregation.

Endothelial Response to Exogenous Triggers for ACS

There are multiple triggers that may initiate the events of ACS and SCD, among them mental stress and an acute elevation of blood pressure associated with sympathetic activation. Previous epidemiological studies demonstrated the association between stressful situations and cardiac events. The vascular response to mental stress is regulated by the endothelium, and its dysfunction may result in coronary vasoconstriction in response to mental stress and in myocardial ischemia. The induction of mental stress may result in prolonged endothelial dysfunction, which may be mediated by release of endothelin-1. Thus, one of the mechanisms by which endothelial dysfunction may lead to cardiovascular events is by mediating the systemic vascular response to exogenous stimuli.

One of the main factors associated with ACS and SCD is cigarette smoking, and both active and passive environmental cigarette smoking contribute to acute clinical events. The underlying mechanisms by which cigarette smoking may lead to ACS and sudden death may be multifactorial and may involve inflammation, thrombosis, and vasoconstriction, all of which characterize endothelial dysfunction. Indeed, cigarette smoking was found to be associated with coronary endothelial dysfunction and with increased monocyte–endothelial cell adhesion. Thus, endothelial dysfunction may be one of the main mechanisms by which cigarette smoking may mediate cardiovascular events.

Heart Failure

The coronary endothelium regulates coronary blood flow of the epicardial and intramyocardial microcirculation. The increase in coronary blood flow in response to an increase in myocardial demand, such as exercise, is at least in part endothelium-dependent. Indeed, in humans without significant coronary artery disease, coronary endothelial dysfunction is associated with perfusion abnormalities on exercise thallium scan. Moreover, induction of coronary endothelial dysfunction is associated with myocardial perfusion defects, and patients with syndrome X may exhibit subendocardial perfusion abnormalities. Furthermore, the myocardial blood flow response to increased demand is a strong independent predictor for the progression of heart failure.

Thus, it may be speculated that endothelial dysfunction may lead to repeated episodes of myocardial ischemia and small infarcts that ultimately contribute to the development of...
heart failure. This hypothesis is supported by the observations that endothelial dysfunction is present in patients with early asymptomatic\textsuperscript{61} as well as symptomatic\textsuperscript{62} heart failure.

**Treatment Strategies**

There is currently no gold standard treatment for endothelial dysfunction. However, several pharmacological and nonpharmacological approaches have been developed in the past several years. The key pharmacological agents that were described as potential treatment for endothelial dysfunction included statins and ACE inhibitors.\textsuperscript{1} These medications are also known to reduce cardiac events, underscoring the role of endothelial function in cardiovascular events.

Nonpharmacological approaches may also promote preservation of healthy endothelium. Exercise training improves endothelium-dependent vasodilatation both in epicardial coronary vessels and in resistance vessels in patients with coronary artery disease,\textsuperscript{63} and exercise training enhances endothelium-dependent dilation in young men of average fitness.\textsuperscript{64} Moreover, enhanced external counterpulsation enhances peripheral endothelial function, with beneficial clinical effects persisting at 1-month follow-up in patients with symptomatic coronary artery disease.\textsuperscript{65} These may contribute to the benefit of regular exercise in preventing cardiovascular disease.

**Conclusions**

Atherosclerosis and ischemic heart disease are the leading causes of morbidity and mortality in Western society and a worldwide epidemic. Despite the success in reducing the mortality from acute cardiovascular events, the incidence of cardiovascular disease and its complications continues to increase. Compelling scientific evidence suggests that the endothelium plays a seminal role in the regulation of vascular tone, growth, thrombogenicity, and inflammation. Moreover, it is evident that the presence of endothelial dysfunction is associated with cardiovascular events. However, there is a void in a prospective randomized trial, which demonstrates that improvement of endothelial function is associated with a reduction in cardiovascular events and attenuation of the disease process. Nevertheless, the present body of evidence clearly indicates that the assessment of endothelial function is continuing to emerge as a key adjuvant diagnostic tool to stratify patients at risk for cardiovascular events.

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