Endothelial Dysfunction
A Marker of Atherosclerotic Risk

Piero O. Bonetti, Lilach O. Lerman, Amir Lerman

Abstract—Endothelial dysfunction is a systemic disorder and a key variable in the pathogenesis of atherosclerosis and its complications. Current evidence suggests that endothelial status is not determined solely by the individual risk factor burden but rather, may be regarded as an integrated index of all atherogenic and atheroprotective factors present in an individual, including known as well as yet-unknown variables and genetic predisposition. Endothelial dysfunction reflects a vascular phenotype prone to atherogenesis and may therefore serve as a marker of the inherent atherosclerotic risk in an individual. In line with this hypothesis, dysfunction of either the coronary or peripheral vascular endothelium was shown to constitute an independent predictor of cardiovascular events, providing valuable prognostic information additional to that derived from conventional risk factor assessment. Interventions like risk factor modification and treatment with various drugs, including statins and angiotensin-converting enzyme inhibitors, may improve endothelial function and thereby, potentially prognosis. Hence, given its reversibility and granted the availability of a diagnostic tool to identify patients at risk and to control the efficacy of therapy in clinical practice, endothelial dysfunction may be an attractive primary target in the effort to optimize individualized therapeutic strategies to reduce cardiovascular morbidity and mortality. (Arterioscler Thromb Vasc Biol. 2003;23:168-175.)

Key Words: endothelial dysfunction ■ atherosclerosis ■ risk factor ■ cardiovascular event ■ prognosis

During the last 2 decades, it has become evident that the vascular endothelium is an active paracrine, endocrine, and autocrine organ that is indispensable for the regulation of vascular tone and maintenance of vascular homeostasis (Table 1). Moreover, recent insights into the basic mechanisms involved in atherogenesis indicate that deleterious alterations of endothelial physiology, also referred to as endothelial dysfunction, represent a key early step in the development of atherosclerosis and are also involved in plaque progression and the occurrence of atherosclerotic complications.1,2

Endothelial dysfunction is characterized by a reduction of the bioavailability of vasodilators, in particular, nitric oxide (NO), whereas endothelium-derived contracting factors are increased.3,4 This imbalance leads to an impairment of endothelium-dependent vasodilation, which represents the functional characteristic of endothelial dysfunction. On the other hand, endothelial dysfunction, aside from denoting impaired endothelium-dependent vasodilation, also comprises a specific state of “endothelial activation,” which is characterized by a proinflammatory, proliferative, and procoagulatory milieu that favors all stages of atherogenesis.4,5 Given this relationship between endothelial dysfunction and atherosclerosis, it is likely that the status of endothelial function may reflect the propensity of an individual to develop atherosclerotic disease, and thus, the presence of endothelial dysfunction may serve as a marker of an unfavorable cardiovascular prognosis.

Received August 1, 2002; revision accepted November 22, 2002.

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Arterioscler Thromb Vasc Biol is available at http://www.atvbaha.org

DOI: 10.1161/01.ATV.0000051384.43104.FC
The current review will discuss the role of endothelial dysfunction as a specific marker of individual atherosclerotic risk by addressing (1) the systemic nature of endothelial dysfunction, (2) the relationship between endothelial function and conventional cardiovascular risk factors, (3) the role of endothelial dysfunction as a mediator and independent predictor of cardiovascular events, (4) the clinical significance of therapeutic options to improve endothelial function, and (5) the question of whether endothelial function testing may be of use in targeting therapeutic strategies aimed at reducing cardiovascular risk.

**Endothelial Dysfunction Is a Systemic Disorder**

Since the first description of endothelial dysfunction in atherosclerotic epicardial coronary arteries in 1986 by Ludmer and colleagues,^5^ invasive assessment of coronary endothelial function by quantitative coronary angiography and coronary Doppler flow measurements, along with graded intracoronary infusions of endothelium-dependent vasodilators such as acetylcholine, may be considered the "gold standard" for endothelial function testing. However, during the last decade, other less-invasive or noninvasive techniques for the assessment of endothelial function, including strain-gauge forearm plethysmography in conjunction with intrararterial infusion of endothelium-dependent vasodilators, such as methacholine or acetylcholine, and high-resolution external vascular ultrasound to measure flow-mediated endothelium-dependent dilation (FMD) of the brachial artery during reactive hyperemia, have been developed. These techniques are based on the fact that endothelial dysfunction is not confined to the coronary arteries but rather represents a systemic disorder that also affects peripheral vascular beds, including both conduit arteries and small resistance vessels in the extremities.^7^

Given the systemic nature of endothelial dysfunction, the question arises to whether peripheral vascular function parallels that of the coronary arteries and thus, may serve as a surrogate marker to identify individuals with coronary endothelial dysfunction. Indeed, 2 studies demonstrated a correlation between FMD of the brachial artery and epicardial coronary dilation in response to intracoronary infusion of acetylcholine or ATP, respectively, underscoring the systemic nature of endothelial function.^8^ However, despite its widespread use as a research tool and the recent development of guidelines to standardize its use,^9^ protocols for assessing FMD of the brachial artery still vary among different laboratories and are operator dependent.^4^ This decreases the feasibility of this noninvasive technique as a valuable screening tool for endothelial dysfunction in clinical practice. Thus, there is growing interest in developing a noninvasive, reliable method to assess endothelial function.

**Endothelial Dysfunction and Risk Factors**

Although the association between cardiovascular risk factors and atherosclerotic disease is well documented, the mechanisms by which these risk factors induce lesion formation and lead to events is not entirely defined. The observation that certain individuals do not develop atherosclerotic manifestations despite the presence of several cardiovascular risk factors suggests the existence of a "threshold switch" that, only when activated, translates the risk factor into an unfavorable vascular effect. Given its strategic location and biological properties, the endothelial cell layer that represents a mechanical and biological barrier between the blood and the vascular wall is likely to serve as the "missing link" between any given risk factor and its detrimental vascular effect.

Most if not all risk factors that are related to atherosclerosis and cardiovascular morbidity and mortality, including traditional and nontraditional risk factors, were also found to be associated with endothelial dysfunction. Many of these risk factors, including hyperlipidemia, hypertension, diabetes, and smoking are associated with overproduction of reactive oxygen species or increased oxidative stress. By reacting with
Endothelial Dysfunction and Myocardial Ischemia

Coronary endothelial dysfunction, which is characterized by impaired NO bioavailability, may be associated with myocardial ischemia. Both physical exercise and mental stress, 2 of the main triggers for an increase in myocardial demand, are associated with epicardial coronary endothelial dependant vasodilation. On the other hand, diverse studies indicated an association between the presence of coronary microvascular endothelial dysfunction and angina pectoris in patients with angiographically normal coronary arteries. Zeiher and colleagues demonstrated an association between impaired coronary endothelium-dependent vasodilation and exercise-induced myocardial perfusion defects in patients without hemodynamically relevant coronary artery disease (CAD). We provided evidence for a direct contribution of coronary endothelial dysfunction to myocardial ischemia by demonstrating the appearance of myocardial perfusion defects associated with intracoronary administration of acetylcholine in patients without obstructive CAD. Hence, myocardial ischemia may result from endothelial dysfunction of either epicardial arteries or coronary microvessels.

Endothelial Dysfunction and Acute Coronary Syndromes

Acute complications play a crucial role in delineating the clinical expression of atherosclerotic disease. Endothelial dysfunction may play a fundamental role in the pathogenesis of acute coronary syndromes (ACSs), such as unstable angina and acute myocardial infarction. Plaque destabilization, the detrimental process that predisposes the plaque to rupture, results from a complex interplay of inflammatory effects that involve cellular plaque components and various proinflammatory mediators. Endothelial dysfunction is associated with increased oxidative stress, an important promotor of inflammatory processes. NO may reduce endothelial expression of several inflammatory mediators and adhesion molecules that increase plaque vulnerability, an effect that is mainly mediated 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 owing to its reduced anti-inflammatory potential.
Precipitation of ACSs 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, elevated arterial stiffness49,50 it can be speculated that pulse-wave amplitude is negatively related to arterial stiffness and that endothelial dysfunction is associated with increased arterial stiffness.42 Moreover, vasoconstrictor response to these stimuli is increased by endothelin-1,43 concentrations of which are elevated in the plasma of patients with early and advanced atherosclerosis44,45 as well as in culprit lesions.46

In summary, endothelial dysfunction contributes to enhanced plaque vulnerability, may trigger plaque rupture, and favors thrombus formation and thus may be viewed as an important contributory factor for several aspects of ACSs.

**Endothelial Dysfunction and Prognosis**

Strong evidence of a role for endothelial dysfunction as a marker of atherosclerotic risk stems from several studies investigating the association between the presence of endothelial dysfunction in both the coronary and systemic circulations and prognosis (Table 2). We recently reported the long-term follow-up results of patients without obstructive coronary atherosclerosis who had undergone invasive coronary endothelial function testing.16 When these patients were stratified by their coronary microvascular endothelial function status, cardiac events occurred only in those with coronary endothelial dysfunction during follow-up. These findings were extended to the epicardial coronary arteries by Schächinger and colleagues,47 who reported long-term follow-up results of patients with various stages of CAD in whom epicardial coronary endothelial function was assessed initially. In that study, patients who experienced cardiovascular events affecting the coronary or systemic circulation demonstrated significantly impaired endothelium-dependent epicardial coronary vasoactivity at the initial examination. Moreover, multivariate analysis, which included traditional cardiovascular risk factors and angiographic evidence for atherosclerosis, identified the presence of epicardial coronary endothelial dysfunction as an independent predictor of future cardiovascular events. Recently, the prognostic significance of microvascular and epicardial coronary endothelial dysfunction was confirmed by Halcox and colleagues.17 In this study, patients who underwent coronary endothelial function testing were followed up. Multivariate analysis, which controlled for the presence of CAD and traditional cardiovascular risk factors, revealed that both epicardial and microvascular coronary endothelial dysfunction independently predicted acute cardiovascular events in patients with and without CAD.

First evidence of a prognostic effect of systemic endothelial dysfunction was suggested by data of the prospective “Men born in 1914” cohort, who underwent venous occlusion plethysmography of the calf between 1968 and 1970.48 In this study, a low pulse-wave amplitude during reactive hyperemia was associated with a higher cardiac event rate and a higher all-cause mortality rate during 21 years of follow-up. Given that pulse-wave amplitude is negatively related to arterial stiffness and that endothelial dysfunction is associated with increased arterial stiffness,49,50 it can be speculated that patients with a low pulse-wave amplitude had peripheral endothelial dysfunction. Recently, 3 studies have shed more light on the relation between the presence of systemic endothelial dysfunction and an adverse outcome. Perticone and colleagues51 followed up hypertensive patients who initially underwent endothelial function testing of the forearm vasculature by strain-gauge plethysmography in connection with intra-arterial infusion of graded doses of acetylcholine for an average of 31.5 months. During follow-up, cardiac, cerebrovascular, and peripheral vascular major adverse events occurred, whereby the risk to experience such an event depended on the magnitude of impairment of endothelial function of the forearm vasculature. In addition, multivariate analysis revealed the presence of severe peripheral endothelial dysfunction as an independent predictor of cardiovascular

### Table 2: Studies on the Prognostic Effect of Coronary and Peripheral Endothelial Dysfunction

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Population</th>
<th>Assessment of End Function</th>
<th>Mean Follow-Up, months</th>
<th>Cardiovascular Event Rate, %</th>
<th>End Dysfunction as an Independent Predictor of Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al Suwaidi et al46</td>
<td>157</td>
<td>Pts without significant coronary stenoses</td>
<td>Coronary microvasculature</td>
<td>28</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Schächinger et al47</td>
<td>147</td>
<td>Pts with various stages of CAD</td>
<td>Coronary macrovasculature</td>
<td>80</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Halcox et al47</td>
<td>308</td>
<td>Pts with/without CAD</td>
<td>Coronary micro-/macrovasculature</td>
<td>46</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Perticone et al47</td>
<td>225</td>
<td>Pts with untreated hypertension</td>
<td>Forearm microvasculature</td>
<td>32</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Heitzer et al47</td>
<td>281</td>
<td>Pts with CAD</td>
<td>Forearm microvasculature</td>
<td>54</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Golce et al47</td>
<td>187</td>
<td>Pts undergoing vascular surgery</td>
<td>Brachial artery</td>
<td>1</td>
<td>8</td>
<td>32</td>
</tr>
</tbody>
</table>

End indicates endothelial; Pts, patients.
events in this hypertensive population. In line with these findings, Heitzer and colleagues, using the same methods, identified the presence of endothelial dysfunction in the forearm microvasculature as an independent predictor for an increased long-term cardiovascular risk in patients with documented CAD. Finally, Gokce and colleagues measured FMD of the brachial artery in response to reactive hyperemia in 187 patients who subsequently underwent vascular surgery and prospectively followed up these patients for 30 days after surgery. During follow-up, a total of 45 patients experienced a postoperative cardiovascular event. Multivariate analysis identified impaired brachial FMD as an independent predictor of adverse short-term postoperative outcome.

Taken together, these studies clearly indicate an association between the presence of both coronary and systemic endothelial dysfunction and an increased risk for future cardiovascular events, further underscoring the systemic nature of endothelial dysfunction and its impact on future cardiovascular morbidity and mortality. Interestingly, the fact that endothelial dysfunction of coronary and peripheral microvessels as well as that of the brachial artery, which hardly ever develops atherosclerotic lesions, is linked to an adverse outcome indicates that additional local or genetic factors play a fundamental role for the transition from a mere atherogenic milieu to overt disease. However, the prognostic impact of endothelial dysfunction in peripheral, easily accessible arteries suggests that assessment of peripheral endothelial function may represent an additional means for risk stratification in patients at possible risk for cardiovascular events, as soon as standardized and reproducible techniques for peripheral endothelial function testing become available.

Therapeutic Strategies to Improve Endothelial Function
Endothelial dysfunction is a reversible disorder, and strategies aimed at reducing cardiovascular risk factors, such as cholesterol lowering, antihypertensive therapy, smoking cessation, estrogen replacement therapy in postmenopausal women, supplementation with folic acid, supplementation with folic acid, and physical exercise, also translate into an improvement in endothelial health, further supporting the association between risk factors and endothelial dysfunction. Moreover, the observation that several pharmacological interventions that improve endothelial function are associated with a decrease in cardiovascular events independent of risk factor modification supports the concept that cardiovascular risk factors share a common pathway that leads to endothelial dysfunction, such as oxidative stress.

Although lowering of elevated cholesterol levels by itself is associated with an improvement in endothelial function independent of the therapeutic strategy used, treatment with statins was shown to be particularly effective for this purpose. Notably, a large body of experimental evidence indicates that statins may improve endothelial function by lipid-independent mechanisms, and recent reports suggest that similar statin effects may also exist in humans. These “endothelium-protective” effects may be mediated by the statins’ antioxidant and anti-inflammatory properties and their capability to restore vascular NO bioavailability. Thus, it may be speculated that such properties are likely to contribute to the favorable effect of these compounds on endothelial function and cardiovascular prognosis in both primary and secondary prevention.

Angiotensin-converting enzyme inhibitors (ACEIs) increase NO bioavailability by decreasing the synthesis of angiotensin II and by enhancing serum levels of NO-releasing bradykinin via inhibition of its degradation. Moreover, ACEIs may also enhance the activity of endothelium-derived hyperpolarizing factor under certain conditions. That short- and long-term ACEI administration may indeed translate into an improvement in both coronary and peripheral endothelial function was shown for patients with CAD and/or its risk factors, although the magnitude of this effect may vary depending on the compound used, the presence of risk factors, and genetic variables. In contrast to ACEIs, controversial results have been reported regarding the effect of angiotensin receptor antagonists, which specifically block the effects of angiotensin II on the angiotensin type 1 (AT1) receptor, on endothelial function. Given that increased oxidative stress plays a pivotal role in the pathogenesis of endothelial dysfunction, administration of antioxidants would be expected to be a reasonable strategy to treat this disorder. Indeed, supplementation with antioxidants, such as glutathione, N-acetylcysteine, and, with a few exceptions, vitamin C, vitamin E has been shown to reverse endothelial dysfunction in coronary and peripheral arteries of patients with cardiovascular risk factors and/or overt atherosclerosis. On the other hand, in contrast to in vitro studies and rather unexpectedly, current clinical data regarding the effect of vitamin E supplementation on endothelium-dependent vasoreactivity are inconsistent.

The observation that interventions such as supplementation of the substrate for NO synthesis, l-arginine, and calcium channel blockade with nifedipine were shown to improve endothelial function without altering the risk factor profile further supports the concept that the endothelium serves as the common mediator of the detrimental effect of various cardiovascular risk factors.

Clinical Relevance of Therapeutic Improvement of Endothelial Function
Although various interventions were shown to be associated with improvement in endothelial function, little is currently known about the clinical and prognostic effect of therapeutic improvement in endothelial function. Cholesterol lowering with statins and therapy with ACEIs are also associated with a reduction of myocardial ischemia in patients with documented CAD. Moreover, the improvement in coronary endothelial function by l-arginine supplementation was associated with a reduction in anginal symptoms, suggesting a reduction in myocardial ischemia in patients with coronary endothelial dysfunction. Taken together, these findings point out the clinical significance of therapeutic improvement in coronary endothelial function. In contrast, given the negative results of recent prospective long-term antioxidant supplementation trials and the Heart and Estrogen/progestin Replacement Study (HERS), direct proof for a causal relation between improvement in endothelial function and reduction in cardiovascular events has yet to be established.
Conclusions

Endothelial dysfunction is a systemic disorder and a critical element in the pathogenesis of atherosclerotic disease and its complications. Growing evidence suggests that the individual burden of currently known cardiovascular risk factors is not the only determinant of endothelial function. Rather, endothelial integrity depends on the balance of all cardiovascular risk factors and vasculoprotective elements in a given individual, including yet-unknown variables and genetic predisposition. Endothelial dysfunction may be regarded as a marker of the inherent atherosclerotic risk in an individual. In line with this hypothesis, the presence of endothelial dysfunction in either the coronary or the peripheral circulation was shown to be an independent predictor of an adverse cardiovascular outcome, providing prognostic information additional to that derived from conventional risk factor assessment.

Given the important role of endothelial dysfunction for the development and progression of atherosclerosis, it seems attractive to consider endothelial dysfunction, aside from the treatment of established cardiovascular risk factors, as a primary therapeutic target in the prevention of atherosclerotic disease. However, before endothelial functional status can be recommended as a primary end point to guide the use of appropriate therapeutic strategies to reduce cardiovascular risk, 2 prerequisites must be fulfilled.1 Such a therapeutic strategy would presume the availability of an appropriate diagnostic tool that allows identification of candidate patients. To be considered clinically useful, such a technique, aside from having a high sensitivity and specificity for the detection of endothelial dysfunction, would ideally have to be noninvasive, easy to perform, accurate, reproducible, and inexpensive.2 There must be direct evidence that therapeutic improvement in endothelial function translates into lower cardiovascular event rates. Thus, to date, targeting the established and modifiable cardiovascular risk factors remains the primary strategy to improve endothelial function and prognosis in individuals at risk for atherosclerotic disease. However, as techniques to reliably assess endothelial function will become available, targeting endothelial dysfunction to optimize individualized risk reduction strategies might become reality in the future.

Acknowledgments

This work was supported by the National Institutes of Health (grant RO1 HL-63911), the Miami Heart Research Institute, the Mayo Foundation, the Margarete und Walther Lichtenstein Stiftung Basel, Switzerland, and the Freiwillige Akademische Gesellschaft Basel, Switzerland. Dr Amir Lerman is an Established Investigator of the American Heart Association.

References


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Arterioscler Thromb Vasc Biol. 2003;23:168-175; originally published online December 12, 2002;
doi: 10.1161/01.ATV.0000051384.43104.FC
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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