Testing Endothelial Vasomotor Function
Nitric Oxide, a Multipotent Molecule
Peter Ganz, MD; Joseph A. Vita, MD

The initial description in 1980 by Furchgott and Zawadzki of endothelium-derived vasodilator factor has stimulated more than 2 decades of intense research to delineate the basic biology of the endothelium and its importance in the clinical setting. Endothelium-derived vasodilator factor has been identified as nitric oxide (NO). It is formed in endothelial cells from the amino acid L-arginine by endothelial isoform of NO synthase (eNOS), which is the product of the NOS3 gene. In addition to producing NO constitutively, the enzyme may be stimulated to increase NO synthesis by a variety of physiological agonists, shear stress, and pharmacological agents. Although discovered as a vasodilator, NO mediates many of the protective functions of the endothelium. It limits vascular recruitment of leukocytes by inhibiting the expression of proinflammatory cytokines, chemokines, and leukocyte adhesion molecules. In addition to producing NO constitutively, the enzyme may be stimulated to increase NO synthesis by a variety of physiological agonists, shear stress, and pharmacological agents. Although discovered as a vasodilator, NO mediates many of the protective functions of the endothelium. It limits vascular recruitment of leukocytes by inhibiting the expression of proinflammatory cytokines, chemokines, and leukocyte adhesion molecules. It inhibits vascular smooth muscle proliferation and platelet adhesion and aggregation. NO also inhibits the production of tissue factor, a molecule that plays a critical role in the propensity of disrupted atherosclerotic plaques to cause intravascular thrombosis. In the setting of risk factors and experimental atherosclerosis, loss of the biological activity of endothelium-derived NO is accompanied by other alterations in endothelial phenotype that further increase the propensity for vasoconstriction, thrombosis, inflammation, and cellular proliferation in the vascular wall. Thus, endothelial dysfunction has the potential to contribute to key events in the course of human atherosclerosis.

NO in Health and Atherosclerosis: Relationship With Risk Factors and Hemodynamic Stress
The experimental observations of Furchgott and others have stimulated translational research to elucidate the importance of endothelium-dependent vasodilation in human coronary atherosclerosis. Accordingly, Ludmer and colleagues administered the endothelium-dependent dilator acetylcholine into the coronary arteries of subjects undergoing cardiac catheterization. Acetylcholine induced dilation of normal epicardial coronary arteries but induced abnormal vasoconstriction, indicative of endothelial dysfunction, in patients with angiographic evidence of atherosclerosis. The concept of endothelial vasodilator dysfunction in atherosclerotic human coronary arteries was reinforced by similar findings when other stimuli to NO release were tested, including flow-mediated dilation, sympathetic activation, serotonin, and adenosine diphosphate.

The loss of endothelium-dependent dilation occurs in the earliest stages of atherosclerosis. In fact, it has been linked to each of the known atherogenic risk factors, including several forms of dyslipidemia, hypertension, diabetes mellitus, cigarette smoking, aging, menopause, family history of premature atherosclerosis, and hyperhomocysteinemia. The endothelium is a direct, sensitive target for the damaging effects of atherogenic risk factors, as evidenced from the experimental introduction of risk factors into healthy subjects. For example, elevation of blood homocysteine by administration of its precursor methionine, generation of lipoprotein remnant
Assessment of Endothelium-Dependent Vasodilator Function in Humans

As originally described by Ludmer and colleagues, endothelial function in human coronary arteries can be assessed by measuring the vasomotor responses of epicardial arteries by quantitative coronary angiography in response to graded concentrations of acetylcholine or other agonists. Endothelial function in coronary resistance vessel responses can be assessed at the same time by Doppler flow measurements. This method has been considered the “gold standard” against which other tests of endothelial function have been compared. It has been particularly useful for developing a framework that relates disturbed coronary pathophysiology to myocardial ischemia in patients with coronary artery disease. This invasive method is of necessity restricted to patients undergoing clinically indicated cardiac catheterization.

Endothelial function of forearm resistance vessels can be assessed by measurement of forearm blood flow using strain-gauge plethysmography in conjunction with intra-arterial infusion of endothelium-dependent agonists and selective pharmacological probes. This approach has been used primarily in studies intended to elucidate the basic mechanisms that underlie endothelial dysfunction in humans. The general applicability of this technique to broader populations is limited by the requirement for an intra-arterial catheter.

Assessment of endothelium-dependent, flow-mediated dilatation of the brachial artery using high-resolution ultrasound has provided an entirely noninvasive approach to evaluating endothelial function. This technique uses increased hemodynamic shear stress during reactive hyperemia as a stimulus for the release of NO. The ultrasound approach has permitted studies of endothelial function in populations of asymptomatic subjects in whom cardiac catheterization is not indicated. The same atherogenic risk factors that impair coronary endothelial function similarly affect endothelial function in brachial arteries. However, the concordance between coronary and brachial endothelial responses when both tests are performed in the same patients is only modest. Moreover, the magnitude of the flow-mediated dilation depends on the specific protocol used to elicit reactive hyperemia, which differs from center to center at the present time. Finally, the methodology is associated with a relatively poor signal-to-noise ratio, which reflects variability in brachial artery size and the current resolution of vascular ultrasound. Despite these limitations, the methodology has proven to be particularly valuable for comparing different populations of patients from single centers and for assessing responses to therapeutic interventions over time.

Endothelial Function and Clinical Outcomes

The postulated antiatherogenic role of NO has been supported by clinical studies. Higher rates of myocardial ischemia or infarction have been reported in humans with polymorphisms of eNOS that reduce the activity of the enzyme. Among human cardiac transplant recipients, coronary endothelial dysfunction after transplantation has been associated with accelerated coronary arteriosclerosis.

Several studies have investigated whether endothelial function testing predicts clinical complications associated with atherosclerosis (Table 1). Using several different methods to test endothelial function in coronary and peripheral arteries, patients with endothelial dysfunction had a far greater incidence of adverse cardiovascular events in follow-up compared with patients with preserved endothelial function. This ability of endothelial function testing to predict events was independent of other known risk factors. Although intriguing, these observations have definite limitations. They are largely a retrospective examination of clinical outcomes in patients enrolled in various research protocols in a few laboratories and hence may not be applicable to the population at large. Moreover, as the number of events in each study is small, composite end points consisting of a hard and a soft end point typically have been used. Nonetheless, the totality and consistency of these studies suggest that assessment of endothelial function has the capacity to provide useful prognostic information about future cardiovascular events.

Therapeutic Interventions and Endothelial Function

Because of the pivotal role that endothelial dysfunction plays in atherosclerosis and its complications, numerous strategies of reversing endothelial dysfunction have been investigated. Short-term studies have shown that mechanistically diverse interventions improve endothelial function, including...
correction of lipid abnormalities, inhibition of angiotensin-converting enzyme or angiotensin II receptor, smoking cessation, exercise, and various dietary interventions. Interestingly, all of these interventions have also been shown to reduce cardiovascular events in clinical outcome studies.

During drug development, clinical outcome trials typically require thousands of patients and many years to complete and are associated with prohibitive costs. Hence, surrogate end points such as endothelial function testing have had much appeal in helping to decide which drugs to include in large clinical trials. The observation that an intervention improves endothelial function in a group of patients suggests that the intervention will also reduce cardiovascular risk and holds the promise that endothelial function testing may differentiate responders to treatment from nonresponders. In support of such a role for endothelial function testing, Modena and colleagues32 observed that improvement in brachial artery flow-mediated dilation after initiation of antihypertensive therapy coincided with a reduction in cardiovascular risk compared with patients with persistent endothelial dysfunction. However, that study lacked a standardized intervention, and further studies will be required to confirm the utility of endothelial function testing for this purpose.

Finally, endothelial function does not always correctly predict long-term outcome. For example, hormonal replacement therapy in postmenopausal women is consistently associated with improved endothelial function in peripheral and coronary arteries,33 but primary and secondary prevention clinical trials have proven negative. Given the complex causal mechanisms of atherosclerosis and the diverse effects of potential interventions, it is likely that no single surrogate end point will be completely predictive of clinical outcome. Accordingly, drug development in the field of atherosclerosis will have to rely on a broad panel of surrogate end points that test the impact of therapy on each key aspect of this disease, including endothelial function, inflammation, thrombosis, and plaque regression.

### Endothelial Function Testing in the Coronary Risk Assessment of Generally Healthy Subjects

Another area of great interest is the potential use of endothelial function to stratify risk in individual subjects. Traditional and newly recognized risk factors account for only a portion of estimated risk for cardiovascular events such as myocardial infarction or coronary heart disease death. It is likely that genetic factors and other unrecognized environmental factors also play a role. Because the endothelium may be a target that integrates the damaging effects of the traditional and unknown risk factors, it has been proposed as a potential “barometer” of atherosclerosis risk,34 and as such, studying endothelial function may guide risk assessment and therapy for individuals. C-reactive protein, a biomarker of inflammation, recently received a limited endorsement to serve in that capacity.35

Routine use of a biomarker for screening has profound implications for healthcare benefits and costs. Accordingly, each potential biomarker must pass rigorous tests, such as those we propose in Table 2. Currently, invasive and noninvasive endothelial function testing may reasonably be used to investigate mechanisms of vascular disease and gain insight into the potential utility of new therapies in studies involving groups of patients. However, endothelial function testing does not meet most of the criteria in Table 2 as a biomarker for use in individual patients, so much work remains. Fortunately, simpler methods for endothelial function testing are currently in development and may prove helpful in that regard.

### Table 1. Clinical Studies Supporting the Prognostic Value of Endothelial Vasomotor Function Testing

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Vascular Bed</th>
<th>Test of Endothelial Function</th>
<th>No. of Patients</th>
<th>Follow-Up, mo</th>
<th>Clinical Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al Suwaidi et al45</td>
<td>CAD</td>
<td>Coronary resistance vessels</td>
<td>Acetylcholine</td>
<td>157</td>
<td>28</td>
<td>MI, cardiovascular death, revascularization, CHF</td>
</tr>
<tr>
<td>Schächinger et al57</td>
<td>CAD</td>
<td>Epicardial coronary arteries</td>
<td>Acetylcholine and flow-mediated dilation</td>
<td>147</td>
<td>92</td>
<td>MI, cardiovascular death, revascularization, unstable angina, ischemic stroke</td>
</tr>
<tr>
<td>Halcox et al56</td>
<td>CAD</td>
<td>Epicardial coronary arteries and resistance vessels</td>
<td>Acetylcholine</td>
<td>308</td>
<td>46</td>
<td>MI, cardiovascular death, unstable angina, stroke</td>
</tr>
<tr>
<td>Perticone et al59</td>
<td>Hypertension</td>
<td>Forearm resistance vessels</td>
<td>Acetylcholine</td>
<td>225</td>
<td>32</td>
<td>Cardiac, cerebrovascular, peripheral vascular</td>
</tr>
<tr>
<td>Heitzer et al60</td>
<td>CAD</td>
<td>Forearm resistance vessels</td>
<td>Acetylcholine</td>
<td>281</td>
<td>54</td>
<td>MI, cardiovascular death, ischemic stroke, revascularization</td>
</tr>
<tr>
<td>Neunteufel et al61</td>
<td>CAD</td>
<td>Brachial arteries</td>
<td>Flow-mediated dilation</td>
<td>73</td>
<td>60</td>
<td>MI, revascularization</td>
</tr>
<tr>
<td>Gokce et al65</td>
<td>PAD</td>
<td>Brachial arteries</td>
<td>Flow-mediated dilation</td>
<td>187</td>
<td>1</td>
<td>Cardiovascular death, MI, unstable angina, stroke</td>
</tr>
<tr>
<td>Modena et al62</td>
<td>Hypertension, postmenopausal</td>
<td>Brachial arteries</td>
<td>Flow-mediated dilation</td>
<td>400</td>
<td>67</td>
<td>Cardiovascular events</td>
</tr>
<tr>
<td>Gokce et al65</td>
<td>PAD</td>
<td>Brachial arteries</td>
<td>Flow-mediated dilation</td>
<td>199</td>
<td>14</td>
<td>Cardiovascular death, MI, unstable angina, stroke</td>
</tr>
<tr>
<td>Targonski et al64</td>
<td>Risk factors, but normal coronary arteries</td>
<td>Coronary resistance vessels</td>
<td>Acetylcholine</td>
<td>503</td>
<td>16</td>
<td>Cerebrovascular events</td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease; PAD, peripheral arterial disease; and MI, myocardial infarction.
Table 2. Assessment of Cardiovascular Risk in Apparently Healthy Individuals: Optimal Characteristics for Proposed New Tests

<table>
<thead>
<tr>
<th>Nature of the test</th>
<th>Ability to predict risk</th>
<th>Relationship to established tests of risk</th>
<th>Function as a surrogate end point</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Test should be simple, noninvasive, commercially available, and cost-effective.</td>
<td>• Ability to predict risk should be established in prospective studies.</td>
<td>• New test should add to the predictive value of established tests.</td>
<td>• New test should be useful as a screen for drug development.</td>
</tr>
<tr>
<td>• Test should be reproducible and standardized internationally.</td>
<td>• Test should have population norms to guide results.</td>
<td>• New test should be particularly useful in refining the risk of medium- or low-risk subjects.</td>
<td>• New test should identify individual patients who will benefit from therapy.</td>
</tr>
</tbody>
</table>

Summary

Basic observations by Furchgott and others have stimulated widespread interest in investigations of endothelial function in humans. This “translational research” has come to fruition. Much progress has been made in elucidating the biological mechanisms of human endothelial dysfunction, its relationship to atherosclerosis, and its clinical manifestations. Evidence for the importance of endothelial function has been strengthened by studies that relate endothelial dysfunction to future clinical events. Endothelial function testing may provide a useful target in the discovery and development of new therapies for the pharmaceutical industry. Further research is needed to establish endothelial function testing in the risk stratification and decisions regarding treatment of generally healthy subjects.

Acknowledgments

Dr Ganz’s work is supported by National Institutes of Health grants P50 HL–8743 and 1P50–HL–56985. Dr Vita’s work is supported by National Institutes of Health grants HL55993, HL60886, HL70100, and HL/AI64753.

References


Testing Endothelial Vasomotor Function: Nitric Oxide, a Multipotent Molecule
Peter Ganz and Joseph A. Vita

Circulation. 2003;108:2049-2053
doi: 10.1161/01.CIR.000089507.19675.F9
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/108/17/2049

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located,
click Request Permissions in the middle column of the Web page under Services. Further information about
this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/