Atherosclerosis begins in childhood, progresses silently through a long preclinical stage, and eventually manifests clinically, usually from middle age. Over the last 30 years, it has become clear that the initiation and progression of disease, and its later activation to increase the risk of morbidity events, depends on profound dynamic changes in vascular biology. The endothelium has emerged as the key regulator of vascular homeostasis, in that it has not merely a barrier function but also acts as an active signal transducer for circulating influences that modify the vessel wall phenotype. Alteration in endothelial function precedes the development of morphological atherosclerotic changes and can also contribute to lesion development and later clinical complications.

Appreciation of the central role of the endothelium throughout the atherosclerotic disease process has led to the development of a range of methods to test different aspects of its function, which include measures of both endothelial injury and repair. These have provided not only novel insights into pathophysiology, but also a clinical opportunity to detect early disease, quantify risk, judge response to interventions designed to prevent progression of early disease, and reduce later adverse events in patients.

The present review summarizes current understanding of endothelial biology in health and disease, the strengths and weaknesses of current testing strategies, and their potential applications in clinical research and patient care.

**Endothelium in Normal Vascular Homeostasis**

Although only a simple monolayer, the healthy endothelium is optimally placed and is able to respond to physical and chemical signals by production of a wide range of factors that regulate vascular tone, cellular adhesion, thromboresistance, smooth muscle cell proliferation, and vessel wall inflammation. The importance of the endothelium was first recognized by its effect on vascular tone. This is achieved by production and release of several vasoactive molecules that relax or constrict the vessel, as well as by response to and modification of circulating vasoactive mediators such as bradykinin and thrombin. This vasomotion plays a direct role in the balance of tissue oxygen supply and metabolic demand by regulation of vessel tone and diameter, and is also involved in the remodeling of vascular structure and long-term organ perfusion.

The pioneering experiments of Furchgott and Zawadzki first demonstrated an endothelium-derived relaxing factor that was subsequently shown to be nitric oxide (NO). NO is generated from L-arginine by the action of endothelial NO synthase (eNOS) in the presence of cofactors such as tetrahydrobiopterin. This gas diffuses to the vascular smooth muscle cells and activates guanylate cyclase, which leads to cGMP-mediated vasodilatation. Shear stress is a key activator of eNOS in normal physiology, and this adapts organ perfusion to changes in cardiac output. In addition, the enzyme may be activated by signaling molecules such as bradykinin, adenosine, vascular endothelial growth factor (in response to hypoxia), and serotonin (released during platelet aggregation). The endothelium also mediates hyperpolarization of vascular smooth muscle cells via an NO-independent pathway, which increases potassium conductance and subsequent propagation of depolarization of vascular smooth muscle cells, to maintain vasodilator tone. The endothelium-derived hyperpolarizing factors involved in this process are only partially understood (such as the cytochrome-derived factors and possibly C-type natriuretic peptide), and may differ between vascular beds. However, it is well recognized that Endothelium-Derived Hyperpolarizing Factor can compensate for loss of NO-mediated vasodilator tone, particularly in the microcirculation, and this appears important when NO bioavailability is reduced.

Prostacyclin, derived by the action of the cyclooxygenase system, is another endothelium-derived vasodilator that acts independently of NO. Although it may contribute to some of the other regulatory roles of the endothelium, it appears to have a more limited role in the maintenance of vasodilator tone in humans.

The endothelium modulates vasomotion, not only by release of vasodilator substances, but also by an increase in constrictor tone via generation of endothelin and vasoconstrictor prostanoids, as well as via conversion of angiotensin I to angiotensin II at the endothelial surface. These vasoconstrictor agents predominantly act locally, but may also exert some systemic effects and have a role in the regulation of arterial structure and remodeling.
Endothelial Activation and Atherosclerosis

What is generally referred to as endothelial dysfunction should more appropriately be considered as endothelial activation, which may eventually contribute to arterial disease when certain conditions are fulfilled. Endothelial activation represents a switch from a quiescent phenotype toward one that involves the host defense response (Figure 1). Indeed, most cardiovascular risk factors activate molecular machinery in the endothelium that results in expression of chemokines, cytokines, and adhesion molecules designed to interact with leukocytes and platelets and target inflammation to specific tissues to clear microorganisms.

The fundamental change involved in this process is a switch in signaling from an NO-mediated silencing of cellular processes toward activation by redox signaling. Reactive oxygen species (ROS), in the presence of superoxide dismutase, lead to generation of hydrogen peroxide, which, like NO, can diffuse rapidly throughout the cell and react with cysteine groups in proteins to alter their function. However, because of the different chemistry involved, this results in very different consequences, such as phosphorylation of transcription factors, induction of nuclear chromatin remodeling and transcription genes, and protease activation. It is intriguing that eNOS, which normally helps maintain the quiescent state of the endothelium, can switch to generate ROS in appropriate circumstances as part of endothelial activation. This is termed eNOS uncoupling, and results in superoxide formation if the key cofactor tetrahydrobiopterin is not present, or generation of hydrogen peroxide if the substrate L-arginine is deficient. Thus, the ability of eNOS to regulate both the quiescent and activated endothelial phenotype puts this enzyme at the center of endothelial homeostasis.

If endothelial activation and redox signaling are part of normal host defense, it is intriguing to consider the circumstances in which they may contribute to atherogenesis and clinical events. The difference between normal host defense and detrimental cellular activation may well be a consequence of the nature, extent, duration, and combination of the proinflammatory stimuli. For example, we have recently shown a profound but transient reduction of endothelium-dependent dilatation associated with mild childhood infection. This may be adaptive and not necessarily proatherogenic, but could become so if other adverse environmental conditions are also present. These might include risk factors such as hypercholesterolemia, hypertension, and diabetes, as well as other inflammatory conditions, such as periodontitis, which may induce chronic dysregulation of NO and ROS production. All of these environmentally driven mechanisms of endothelial activation are likely to be modulated by genetic factors.

In certain circumstances, chronic production of ROS may exceed the capacity of cellular enzymatic and nonenzymatic antioxidants, and thus contribute to vascular disease by induction of sustained endothelial activation. An important source of ROS is probably the mitochondrion, in which production of ROS and the dismuting capacity of mitochondrial superoxide dismutase are typically carefully balanced during oxidative phosphorylation. This may be disturbed during hypoxia or conditions of increased substrate delivery, such as occurs in obesity-related metabolic disorders or type II diabetes, which are characterized by hyperglycemia and increased circulating free fatty acids. Other important sources of oxidative stress in the endothelium are nicotinamide adenine dinucleotide phosphate oxidases, as well as xanthine oxidase, which have been shown to have increased activity in arteries from patients with coronary disease. Endothelial ROS signaling may be initiated by exposure to inflammatory cytokines and growth factors, and the interaction of the endothelium with leukocytes. Regardless of their source, the interaction between ROS and NO sets up a vicious circle, which results in further endothelial activation and inflammation.

Endothelial Injury and Repair

Prolonged and/or repeated exposure to cardiovascular risk factors can ultimately exhaust the protective effect of endog-
enous antiinflammatory systems within endothelial cells. As a consequence, the endothelium not only becomes dysfunctional, but endothelial cells can also lose integrity, progress to senescence, and detach into the circulation (Figure 2). Circulating markers of such endothelial cell damage include endothelial microparticles derived from activated or apoptotic cells, and whole endothelial cells. These markers have been found to be increased in both peripheral and coronary atherosclerosis disease, as well as other inflammatory conditions associated with increased vascular risk such as rheumatoid arthritis and systemic lupus erythematosus. Circulating endothelial microparticles and endothelial cells can be quantified, and may be promising candidates for clinical testing (see below).

Endothelial integrity depends not only on the extent of injury, but also on the endogenous capacity for repair. Two mechanisms by which this process of repair occurs have been recently identified. Adjacent mature endothelial cells can replicate locally, and replace the lost and damaged cells. A recent modeling study suggested that, although local endothelial cells would be sufficient to maintain vascular integrity throughout life in healthy circumstances, in the presence of risk factors, loss of endothelial integrity would rapidly develop if local replication were the only repair mechanism. More recently, it has become clear that circulating endothelial progenitor cells are an alternative mechanism for maintenance and repair of the endothelium. These cells are recruited from the bone marrow, circulate in the peripheral blood, and can differentiate into mature cells with endothelial characteristics. Mobilization of these cells is in part NO-dependent, and may thus be impaired in patients with cardiovascular risk factors. Conversely, factors that have been shown to improve endothelial function and NO bioavailability, such as exercise and statins, have also been shown to have a potent positive effect on endothelial progenitor cell mobilization. Furthermore, recent evidence has indicated that risk factors not only interfere with the recruitment of circulating endothelial progenitor cells, but also with the differentiation and function of these cells. For example, important cellular properties such as migration, adhesion, and formation of tubules in culture conditions can be impaired in the presence of risk factors and atherosclerotic disease. It is intriguing to note that circulating endothelial progenitor cells may differentiate down different lineages, and develop characteristics of other myeloid cells such as macrophages and dendritic cells when exposed to inflammatory cytokine profiles. Thus, circulating endothelial progenitor cell biology may play a major role in the pathogenesis of vascular disease by an effect on both endothelial injury and the capacity for endothelial repair. The importance of the balance between exposure to risk factors and the capacity for repair in the determination of the clinical endothelial phenotype has been highlighted by the demonstration that subjects with increased numbers of circulating endothelial progenitor cells have preserved endothelial function, despite exposure to high levels of risk factors (Figure 3).

Clinical Assessment of Endothelial Function
An improved understanding of the vascular biology of the endothelium has permitted the development of clinical tests that evaluate several of the functional properties of normal and activated endothelium. Ideally, such tests should be

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**Figure 2.** Sustained ROS signaling induces senescence of endothelial cells. Left, This is reflected in detachment of endothelial cells or parts of the endothelial cell membrane (endothelial microparticles). Right, With increasing age and persisting ROS signaling, the capacity of neighboring endothelial cells to repair the endothelial injury is limited, and vascular integrity becomes dependent on the incorporation of circulating progenitor cells.

**Figure 3.** Relationship between risk factor profile, endothelial dysfunction, and circulating endothelial progenitor cells in middle-aged men. A, Association between endothelial progenitor cells and brachial reactivity (FMD). B, Subjects with high endothelial progenitor cells had preserved endothelial function, even in the presence of a high risk factor score. Reproduced from Hill et al with permission from the Massachusetts Medical Society. Copyright © 2003.
Methods for Clinical Assessment of Endothelial Function

<table>
<thead>
<tr>
<th>Technique (Outcome Measure)</th>
<th>Noninvasive</th>
<th>Repeatable</th>
<th>Reproducible*</th>
<th>Reflects Biology</th>
<th>Reversible</th>
<th>Predicts Outcome†</th>
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<td>Cardiac catheterization (change in diameter, change in coronary blood flow)</td>
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<td>PCA (change in reflective index)</td>
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+ indicates supportive evidence in literature; −, insufficient evidence; FMD, flow-mediated dilatation; PWA, pulse wave analysis; PCA, pulse contour analysis; and PAT, pulse amplitude tonometry.

*Reproducibility of PWA, PCA, and PAT has been less extensively investigated than FMD.
†Studies that link PWA, PCA, and PAT to outcome have not yet been reported.
‡FMD is currently the standard for noninvasive assessment of conduit artery endothelial function because there is considerable clinical trial experience, validation, a firm link to biology, and association with cardiovascular events.

Endothelial-Dependent Vasomotion

Endothelial-dependent vasomotion has been the most widely used clinical end point for assessment of endothelial function. Testing involves pharmacological and/or physiological stimulation of endothelial release of NO and other vasoactive compounds, and often a comparison of vascular responses to endothelium-independent dilators such as nitroglycerine. Determination of local NO bioavailability not only reflects its influence on vascular tone, but also the other important functions of this molecule, which include thromboregulation, cell adhesion, and proliferation.

Initial clinical studies of endothelial function were undertaken in the coronary circulation, and involved local infusion of acetylcholine with measurement of the change in vessel diameter by quantitative coronary angiography. This approach is a direct clinical analog of Furchgott and Zawadzki’s original experiment. Acetylcholine releases NO from vessels with an intact endothelium, which leads to vasodilation, but causes vasoconstriction in subjects with endothelial dysfunction, as a result of a direct muscarinic smooth muscle vasoconstrictor effect. Doses that result in final blood concentrations in the range of $10^{-8}$ to $10^{-5}$ mol are the most appropriate for assessment of the physiological range of responses. Subsequently, these methods have been refined with use of the Doppler flow wires to measure resistance vessel function. Responses to a wide range of endothelial agonists that include substance P, adenosine, and bradykinin have also been measured, as well as physiological responses to cold-pressor testing and flow-mediated dilatation (FMD) of proximal conduit arteries as a result of distal infusion of adenosine. In addition, use of specific NO antagonists such as L-NMMA has defined the contribution of NO to these vasomotor responses. These studies have provided important insights into the vascular effects of risk factors and the potential reversibility of endothelial dysfunction in response to interventions such as statins and angiotensin-converting enzyme inhibitors.

Although these tests directly assess the coronary circulation, their invasive nature limits their use to patients with advanced disease, and precludes repeated testing during serial follow-up. Because endothelial dysfunction is a systemic process, however, a less invasive approach has been developed that utilizes the same principles of local infusion of pharmacological probes and measurement of changes in forearm resistance vessel tone by venous occlusion plethysmography. This has provided an opportunity to evaluate endothelial pathophysiology during the preclinical stage of disease by use of appropriate agonists and antagonists with construction of dose-response curves. A correlation between acetylcholine responses in the coronary circulation and in the forearm has been demonstrated. Venous occlusion plethysmography has been widely used, but it is invasive in that it requires arterial cannulation. This limits its repeatability, and prohibits its use in larger studies. Results are also difficult to standardize because baseline resistance vessel tone is variable, and testing protocols and set-up differ between research laboratories. The clinical relevance to atherosclerosis is also uncertain because microvascular pathophysiology does not necessarily reflect changes in the conduit arteries that are particularly predisposed to develop disease.

For all these reasons, we reported in 1992 a noninvasive ultrasound-based test to assess conduit artery vascular function in the systemic circulation (Figure 4). In this method, brachial artery diameter is measured before and after an increase in shear stress that is induced by reactive hyperemia (FMD). When a sphygmomanometer cuff placed on the forearm distal to the brachial artery is inflated to 200 mm Hg and subsequently released 4 to 5 minutes later, FMD occurs predominantly as a result of local endothelial release of NO.
As in the coronary circulation, this brachial artery response can be contrasted to the endothelium-independent dilator response to sublingual nitroglycerine. This method is technically demanding, but can be standardized to yield reproducible results that correlate with coronary vascular endothelial function. Modern software development has allowed for continuous assessment of arterial diameter and blood flow throughout the whole protocol by use of accurate edge detection algorithms that can be manually edited. It is important to note that variations in technique, such as the position of the occluding cuff and duration of inflation, may produce results that are less representative of local NO activity. Brachial artery FMD has been studied widely in clinical research as it enables serial evaluation of young subjects, including children. It also permits testing of lifestyle and pharmacological interventions on endothelial biology at an early preclinical stage, when the disease process is most likely to be reversible. This test represents the gold standard for clinical research on conduit artery endothelial biology, and has opened up a new field of vascular epidemiology (see below). There are, however, practical challenges that need to be overcome before this technique could be suitable for use in routine clinical practice. These challenges include the need for highly trained operators, the expense of the equipment, and also the care required to minimize the effect of environmental or physiological influences, such as exercise, eating, caffeine ingestion, and important variations in temperature.

FMD is also determined, in part, by the magnitude of postischemic vasodilatation, which makes it also a measure of microcirculatory function.

A number of alternative noninvasive approaches have been developed recently to study vascular biology in the peripheral circulation. These rely on the ability of the β2 agonist salbutamol to reduce arterial stiffness in an NO-dependent manner without significant reduction in blood pressure when given by inhaler at standard clinical doses. Changes in arterial stiffness can be measured with pulse wave analysis by radial artery tonometry or pulse contour analysis by digital photoplethysmography. The changes in augmentation index and reflection index are measured from the peripheral arterial waveform, and a central aortic waveform can be derived from pulse wave analysis data by a transfer function that has been validated in adults. Similarly, reactive hyperemia has been used to elicit changes in conduit artery pulse wave velocity and digital pulse volume that can be measured by oscillometry to identify limb arterial pulse pressure, wave form, timing, and also digital pulse amplitude tonometry. Several of these methods have been validated as measures of NO bioavailability. They have been shown to change with exposure to risk factors and with atherosclerotic disease, and may complement FMD testing. The relative contribution of structural alterations in the vessel wall and endothelial-dependent biology remains uncertain, however. Further validation is required, inclusive of a wider study of their

Figure 4. FMD of the brachial artery. A, Ultrasound probe held in stereotactic clamp with micrometer adjustment. B, Continuous measurement of brachial artery diameter (end-diastolic images obtained every 3 seconds), before, during, and after inflation and release of sphygmomanometer cuff on forearm. C, Relationship of FMD to coronary risk factors in 500 asymptomatic adults. Reproduced from Celermajer et al, copyright © 1994, with permission from the American College of Cardiology Foundation. D, Impact of diet and exercise on FMD in overweight Chinese teenagers over 6 weeks and 1 year. Reproduced from Woo et al with permission from Lippincott, Williams & Wilkins. Copyright © 2004, American Heart Association.
Circulating Markers of Endothelial Function

A broader appreciation of the numerous functions of the endothelium can be obtained by study of the levels of molecules of endothelial origin in circulating blood. These include direct products of endothelial cells that change when the endothelium is activated, such as measures of NO biology, inflammatory cytokines, adhesion molecules, regulators of thrombosis, as well as markers of endothelial damage and repair. Many of these circulating markers are difficult and expensive to measure, and currently are only used in the clinical research setting. In this context, these measures can provide important information regarding mechanisms and severity of endothelial dysfunction in populations, and complement physiological tests of endothelial vascular control. As a result of biological and assay availability and variability, these factors currently have only a very limited role in the assessment of individual patients.

Circulating levels of nitrites and nitrosylated proteins in part reflect endothelial generation of NO, but are difficult to measure and may not always represent endothelial NO production. Specifically, values may be confounded by the formation of adducts from other nitrogen-containing species, other sources of NO, and wide variation in dietary NO. Asymmetric dimethylarginine is an endogenously derived competitive antagonist of NO synthase. Levels are elevated in subjects with risk factors, such as dyslipidemia and hypertension, as well as in subjects with disease states associated with increased risk of atherosclerosis, such as diabetes and renal failure. Increased levels of asymmetric dimethylarginine are associated with a reduction in NO bioavailability in both animal and clinical studies. This increase in asymmetric dimethylarginine is, in part, caused by reduced activity of its breakdown enzyme dimethylarginine dimethylaminohydrolase, which is exquisitely sensitive to the altered cellular redox conditions that accompany risk factors and inflammation. Because asymmetric dimethylarginine levels have been linked to preclinical atherosclerotic disease burden and an adverse outcome, they may well prove to be a useful measure of endothelial status and a potential marker of risk in clinical practice. At present, however, the assay remains challenging and expensive.

Endothelial cell activation leads to increased expression of inflammatory cytokines and adhesion molecules that trigger leukocyte homing, adhesion, and migration into the subendothelial space, which are processes fundamental to atherosclerotic lesion initiation, progression, and destabilization. Well-characterized molecules that can be measured in the circulation with commercial immunoassays include E-selectin, vascular cell adhesion molecule 1, intercellular adhesion molecule 1, and P-selectin. Many of these molecules arise from multiple sources, which are not all clear, but E-selectin is probably the most specific for endothelial cell activation. Levels increase in association with cardiovascular risk factors, and have been associated with structural and functional measures of atherosclerotic disease, as well as with adverse cardiovascular prognosis.

Similarly, the procoagulant consequences of endothelial activation can be measured as a change in the balance of tissue plasminogen activator and its endogenous inhibitor, plasminogen activation inhibitor-1. Furthermore, von Willebrand factor, a largely endothelium-derived glycoprotein, is released into the circulation by activated endothelial cells. This agent has a function in further cellular activation as well as promotion of coagulation and platelet activation, and can be measured relatively easily.

Appreciation that endothelial function reflects the net balance between injury and repair has led to the development of assays to quantify the detachment of mature endothelial cells and derived microparticles to represent the degree of damage, as well as determination of the number and functional characteristics of circulating endothelial progenitor cells to reflect the endogenous repair potential.

Circulating endothelial cells that detach in the context of endothelial activation and loss of integrity can be measured in the circulation by both flow cytometry and a combination of magnetic bead selection and fluorescent microscopy. Mature circulating endothelial cells can be distinguished from circulating endothelial progenitor cells by virtue of their size and the expression of surface markers. The increased levels of circulating endothelial cells in patients with atherosclerotic disease and vascular inflammation suggest a direct relationship between the number of these cells in the peripheral circulation and the extent of endothelial injury. Endothelial microparticles are vesicles formed by the cell membrane after endothelial activation, and their composition can be used to characterize the status of the parent endothelial cell. Elevated circulating microparticles have been seen in a variety of conditions associated with endothelial activation or apoptosis. Their function is unclear, but they may not merely be markers of the state of the endothelium. They may also be diffusible mediators of molecules involved in cell signaling, and thus themselves may be proinflammatory. These observations are exciting, but progress in the understanding of their pathophysiological role as well as in quantification is required before measurement of endothelium-derived microparticles becomes part of clinical practice.

Circulating endothelial progenitor cells can be characterized by the expression of characteristic surface markers, which are detectable by flow cytometry, but because a wide range of hematopoietic progenitor cells, which include abundantly present myeloid precursors, has the potential to adopt an endothelial phenotype, the specificity of these measurements is controversial. Further methods to characterize circulating endothelial progenitor cell biology include quantification of the potential to differentiate into an endothelial cell phenotype, as well as determination of functional characteristics, which include migration toward a chemical stimulus (eg, stromal cell–derived factor 1, vascular endothelial growth factor) adhesion, formation of vascular tubules, and the ability to attenuate ischemia in animal models.

Thus, measurement of circulating endothelial cells and circulating endothelial progenitor cell levels provides a novel and exciting means to follow the determinants of endothelial function.
injury and repair. Although the balance of these 2 cell populations has already been linked to other in vivo measures of endothelial function, and has been shown to associate with future cardiovascular events, these novel measures still remain far from clinical use. Nevertheless, it is likely that important new insights into evolution of disease and potential treatment opportunities will emerge from this rapidly developing field.

**Clinical Applications**

Current evidence suggests that endothelial function is an integrative marker of the net effects of damage from traditional and emerging risk factors on the arterial wall and its intrinsic capacity for repair. This endothelial-dependent vascular biology is critical, not only in the initiation and progression of atherosclerosis, but also in the transition from a stable to an unstable disease state with attendant risks (Figure 5). As a result, study of endothelial function in clinical research has emerged as an important end point that complements measurement of circulating risk factors, imaging techniques for structural arterial diseases burden (such as carotid intima media thickness, intravascular ultrasound, computed tomography), and traditional cardiovascular clinical outcomes.

In patients with established atherosclerosis, disturbed vasomotion associated with endothelial activation may contribute to transient myocardial ischemia and angina pectoris. It is also associated with changes in plaque composition and biology, which may influence plaque stability. It should be appreciated that endothelial function, unlike measures of vessel wall morphology, has intrinsic biological variability, and thus a single measurement, much like blood pressure, may give only a snapshot and limited information. Nevertheless, several studies have shown that a single measurement of endothelial function in both the coronary and peripheral circulation can be of prognostic value in a number of different clinical cohorts, which includes patients with established coronary disease and those with atypical symptoms. A number of issues, however, require further investigation in larger prospective studies. These include the incremental predictive value of this approach above other established risk markers, the applicability to the general population, and the most appropriate testing profile, which might include a combination of tests for circulating endothelial biomarkers and vasomotor responses.

Strategies to reverse endothelial function have now been examined in a wide range of patients with vascular disease. Benefit has been shown with a number of pharmacological interventions, which include drugs that lower lipids and blood pressure, as well as with novel therapies based on new understanding of endothelial biology (eg, dietary supplementation with L-arginine). These have mostly, but not always, shown that recovery of endothelial function occurs in response to strategies known to reduce cardiovascular events. This adds support to the concept that restoration of endothelial function can stabilize the atherosclerotic disease process. Endothelial function testing in patients has also proven useful in the identification of new treatment approaches. Several classes of drugs have been shown to have direct actions on the endothelium that are independent of their effects on cardiovascular risk factors. Examples include the benefit of glitazones on endothelial function in non-diabetic coronary artery disease patients, and those of calcium antagonists in normotensive hypercholesterolemic subjects.

Studies that examine the clinical impact of endothelial function have concentrated on patient cohorts with established coronary and peripheral atherosclerosis, and have mostly shown an independent prognostic impact on cardiovascular outcome. This suggests that vascular function testing may have a role in the clinical phase of atherosclerosis.

The main value, however, of the study of endothelial function and its response to intervention may be earlier in the disease course. In advanced disease, a Pandora’s box of mechanisms has been opened, and outcome may depend on many factors, not all of which can be identified and modified. Indeed, in a small meta-analysis, Witte et al suggested that...
the association between FMD and cardiovascular risk was most obvious in lower-risk populations.\textsuperscript{91}

The development of noninvasive measures to study endothelial function has been a major advance in the evaluation of large cohorts during the long preclinical stage of atherosclerosis. Several clinical reports have shown that endothelial dysfunction develops from the first decade of life in response to genetic and environmental risk factors, and this supports the wealth of experimental data that shows endothelial dysfunction to be on the causal pathway for the initiation and progression of atherosclerosis. We have recently studied endothelial function and measures of arterial stiffness in a cohort of \textgtr;7000 10-year-old children who are part of a large prospective population in which genetic and environmental influences on the emergence of functional vascular epidemiology are being investigated (Avon Longitudinal Study of Parents and Children).\textsuperscript{92} Others have also adopted this "functional vascular epidemiology" approach that uses combinations of structural and functional measures.\textsuperscript{93} In a large population of young adults (The Cardiovascular Risk in Young Finns Study), a strong inverse relationship has been shown between endothelial-dependent FMD and structural arterial disease (by carotid intima media thickness) after multivariable adjustment for traditional risk factors.\textsuperscript{94} This relationship was most striking in those with the worst FMD, which supports a protective role for the quiescent endothelial phenotype, as well as the complementary use of endothelial function testing and structural measurements for characterization of early disease. This relationship has not always been seen in other cohorts of older subjects.\textsuperscript{95}

The importance of the management of not only the clinical phase of atherosclerosis but also early disease is becoming increasingly clear. A recent analysis of the Framingham study has highlighted the impact of early risk factor exposure. Risk factor status at age 50 years had a striking relationship with subsequent cardiovascular outcome and life expectancy in both men and women\textsuperscript{96} (Figure 6). The effect of genetic variants that alter levels of risk factor from childhood has also emphasized the importance of lifetime exposure and the greatly leveraged gains that can be anticipated from early intervention. In the Atherosclerosis Risk In Communities (ARIC) study, a polymorphism of the PCSK9 gene in black subjects resulted in a 28% reduction in LDL cholesterol levels from childhood and an 88% relative reduction in later life.\textsuperscript{97} This concept of lifetime risk management can be modeled to demonstrate the great potential for event-free life prolongation in individual subjects that can result from even modest reductions in risk factors introduced at an early stage.\textsuperscript{98}

It is clear that scientific evaluation of the mechanism and triggers of initiation and progression of early arterial disease can only be performed by use of intermediate phenotype end points, and not by study of morbidity and mortality. This is likely to involve tests of endothelial function together with structural measures.

There is a clear need for clinical testing strategies that refine risk assessment in individual subjects, and in particular identify those at high risk and their response to treatment. Although endothelial function testing has added enormously to the understanding of the atherosclerotic process, it is not yet suitable for screening or individual clinical decision-making.

Endothelial function testing shows great promise in that it reflects important vascular biology, is associated with disease burden and outcome, and responds to interventions, but currently available tests are too difficult, expensive, and variable for routine clinical use. Furthermore, as with other biomarkers used in research, the value in patients will depend on more information about the quantitative relationship between measures of endothelial function and outcome, not merely the associations shown in cohorts. In particular, the technical and biological variability of the various measures of endothelial function will need to be defined in large populations with and without disease. Very few currently fashionable biomarkers fulfill these stringent criteria for clinical use.\textsuperscript{99}

Nevertheless, the ability to measure endothelial function noninvasively has already transformed understanding of the evolution of atherosclerosis. A comprehensive approach that involves measurement of genetic predisposition, risk factors,
endothelial function, and structural arterial disease is likely to be the best way to evaluate new treatment strategies, particularly in the early preclinical phase of disease, during which better management should result in major public health benefits.

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