

STATE-OF-THE-ART PAPER

The Clinical Implications of Endothelial Dysfunction

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Defining new approaches for the prevention and treatment of atherosclerosis is an important priority. Recently, measurement of endothelial function in patients has emerged as a useful tool for atherosclerosis research. Risk factors are associated with impaired endothelial function, and clinical syndromes relate, in part, to a loss of endothelial control of vascular homeostasis. Recent studies have shown that the severity of endothelial dysfunction relates to cardiovascular risk. A growing number of interventions known to reduce cardiovascular risk have been shown to improve endothelial function. This work suggests that studies of endothelial function could be used in the care of patients and as a surrogate marker for the evaluation of new therapeutic strategies. This article will review this growing literature in an effort to evaluate the current clinical utility of endothelial dysfunction. (J Am Coll Cardiol 2003;42:1149–60) © 2003 by the American College of Cardiology Foundation

Measurement of endothelial function in patients has recently emerged as a useful tool for atherosclerosis research. In the setting of cardiovascular disease (CVD) risk factors, the endothelium loses its normal regulatory functions. Clinical syndromes such as stable and unstable angina, acute myocardial infarction, claudication, and stroke relate, in part, to a loss of endothelial control of vascular tone, thrombosis, and the composition of the vascular wall. Recent studies have shown that the severity of endothelial dysfunction relates to the risk for an initial or recurrent cardiovascular event. Finally, a growing number of interventions known to reduce cardiovascular risk also improve endothelial function. This work has prompted speculation that endothelial function serves as a “barometer” for cardiovascular health that can be used for patient care and evaluation of new therapeutic strategies (1). This article will review this growing literature in an effort to evaluate the current clinical utility of assessing endothelial dysfunction.

Normal functions of the endothelium. The endothelium acts to maintain vascular homeostasis through multiple complex interactions with cells in the vessel wall and lumen (reviewed by Gokce et al. [2]). Table 1 lists many of the major factors regulated and elaborated by vascular endothelium. Specifically, the endothelium regulates vascular tone by balancing production of vasodilators, including nitric oxide (NO), and vasoconstrictors. Furthermore, the endo-

thelium controls blood fluidity and coagulation through the production of factors that regulate platelet activity, the clotting cascade, and the fibrinolytic system. Finally, the endothelium has the capacity to produce cytokines and adhesion molecules that regulate and direct the inflammatory process (3).

Pathophysiology of endothelial dysfunction. Under homeostatic conditions, the endothelium maintains normal vascular tone and blood fluidity, and there is little to no expression of pro-inflammatory factors. However, both traditional and novel CVD risk factors initiate a chronic inflammatory process that is accompanied by a loss of vasodilator and anti-thrombotic factors and an increase in vasoconstrictor and pro-thrombotic products. As outlined in Figure 1, risk factors as diverse as smoking, aging, hypercholesterolemia, hypertension, hyperglycemia, and a family history of premature atherosclerotic disease are all associated with an attenuation/loss of endothelium-dependent vasodilation in both adults and children (2,4,5). More recently recognized risk factors such as obesity (6), elevated C-reactive protein (7), and chronic systemic infection (8) also are associated with endothelial dysfunction.

Abnormal vasoreactivity is not the only imbalance present in high-risk individuals. Endothelial cells may adopt a pro-thrombotic phenotype, portending an elevated risk of cardiovascular events in high-risk individuals (9,10). Furthermore, when exposed to certain pathogenic pro-inflammatory stimuli, the endothelium expresses leukocyte chemotactic factors, adhesion molecules, and inflammatory cytokines (11). The precise extent and order in which the normal control mechanisms are affected have yet to be fully elucidated.

The term “endothelial dysfunction” refers to this broad alteration in endothelial phenotype that may contribute to the development and clinical expression of atherosclerosis (12). While the precise mechanisms remain to be eluci-

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Abbreviations and Acronyms

BP	= blood pressure
CAD	= coronary artery disease
CVD	= cardiovascular disease
FMD	= flow-mediated dilation
HRT	= hormone replacement therapy
ICAM	= intercellular adhesion molecule
NO	= nitric oxide
VCAM-1	= vascular cell adhesion molecule-1

dated, endothelial dysfunction appears to participate in a “positive feedback loop” in which inflammatory factors promote monocyte and T-cell adhesion, foam cell formation, extracellular matrix digestion, and vascular smooth muscle migration and proliferation leading to atherosclerotic plaque formation (3,13). Endothelial dysfunction also is relevant to the later stages of the disease, and appears to play a role in acute coronary syndromes (14). Given this possible causal pathway from endothelial dysfunction to atherosclerosis (Fig. 1), numerous methods have been employed to measure endothelial dysfunction in humans.

Methods of evaluating endothelial dysfunction in humans.

While atherosclerosis is associated with a broad alteration in endothelial phenotype, the assessment of endothelium-dependent vasodilation has emerged as an accessible indicator of endothelial health. In particular, stimuli that increase production of endothelium-derived NO have proven useful in assessing endothelium-dependent vasodilation in humans. Such stimuli include increased shear stress from increased blood flow, and receptor-dependent agonists, such as acetylcholine, bradykinin, or substance P. Basal NO release can be assessed using specific inhibitors of NO

Table 1. Normal Functions of the Vascular Endothelium and a Partial List of Factors Elaborated and Regulated by the Endothelium to Maintain Vascular Homeostasis

Maintenance of vascular tone
Nitric oxide
Prostaglandins (prostacyclin [PGI ₂], thromboxane A ₂ [TxA ₂])
Endothelial hyperpolarizing factor
Endothelin-1
Angiotensin II
C-type natriuretic peptide
Balancing blood fluidity and thrombosis
Nitric oxide
Tissue plasminogen activator
Heparins
Thrombomodulin
Prostaglandins
Plasminogen activator inhibitor-1 (PAI-1)
Tissue factor
Von Willibrand's factor
Control of the vascular inflammatory process
Monocyte chemoattractant factor-1 (MCP-1)
Adhesion molecule expression (VCAM-1, ICAM-1, selectins)
Interleukins 1, 6, and 18
Tumor necrosis factor

ICAM-1 = intercellular adhesion molecule-1; VCAM-1 = vascular cell adhesion molecule-1.

synthase, such as NG-monomethyl-L-arginine. Investigators have employed several methods in the evaluation of endothelial function, each with its own advantages and disadvantages (Table 2).

The earliest studies of endothelial control of vasomotion used quantitative coronary angiography to examine the vasomotor responses of the epicardial coronary artery during infusion of acetylcholine (15) or increased blood flow (16). In healthy individuals, the endothelium responds to these stimuli by releasing vasodilator factors, particularly NO. Early studies demonstrated that patients with angiographically proven coronary artery disease (CAD) display impaired flow-mediated dilation (FMD) and a vasoconstrictor response to acetylcholine rather than the normal vasodilator response, likely reflecting loss of NO and unopposed constrictor effects of acetylcholine on vascular smooth muscle (15). More recent studies suggest that acetylcholine-mediated coronary constriction may also result, in part, from enhanced endothelial release of the potent vasoconstrictor endothelin (17).

Invasive studies in the arm involve infusion of endothelium-dependent agonists into the brachial artery and measuring the vasodilator responses of forearm resistance vessels using venous occlusion plethysmography (18). Like studies in the coronary circulation, this approach allows investigators to examine dose-response relations and use specific agonists and antagonists in a more accessible vascular bed. However, the technique requires an arterial catheter and, thus, has limited applicability for large-scale studies or future development as a clinical tool.

Measures of arterial stiffness, including pulse wave velocity and arterial distensibility, are also being investigated as non-invasive means of measuring vascular health (19). Several studies have demonstrated that such measures predict cardiovascular events (20,21). While dynamic factors, such as release of endothelium-derived NO, play a role, arterial stiffness is also highly dependent on fixed structural features of the vascular wall including the degree of fibrosis and calcification (19). Elucidation of the precise relationship between endothelial function and vascular stiffness awaits further study.

Finally, there has been considerable interest in non-invasive examination of endothelium-dependent FMD of the conduit brachial artery using vascular ultrasound (22). This response has been shown to depend in large part on NO synthesis (23,24), but also reflects release of other endothelium-derived vasodilators. Like measures of vascular stiffness, this technique can safely be applied to large and varied groups of patients and can be used to make repeated measurements over time. As in the coronary circulation, endothelial function in the brachial circulation is impaired in the setting of traditional and novel risk factors and responds to interventions known to reduce CVD risk (1). Importantly, studies suggest that endothelial function detected non-invasively in the brachial artery correlates with function in conduit coronary arteries (25). Despite the many

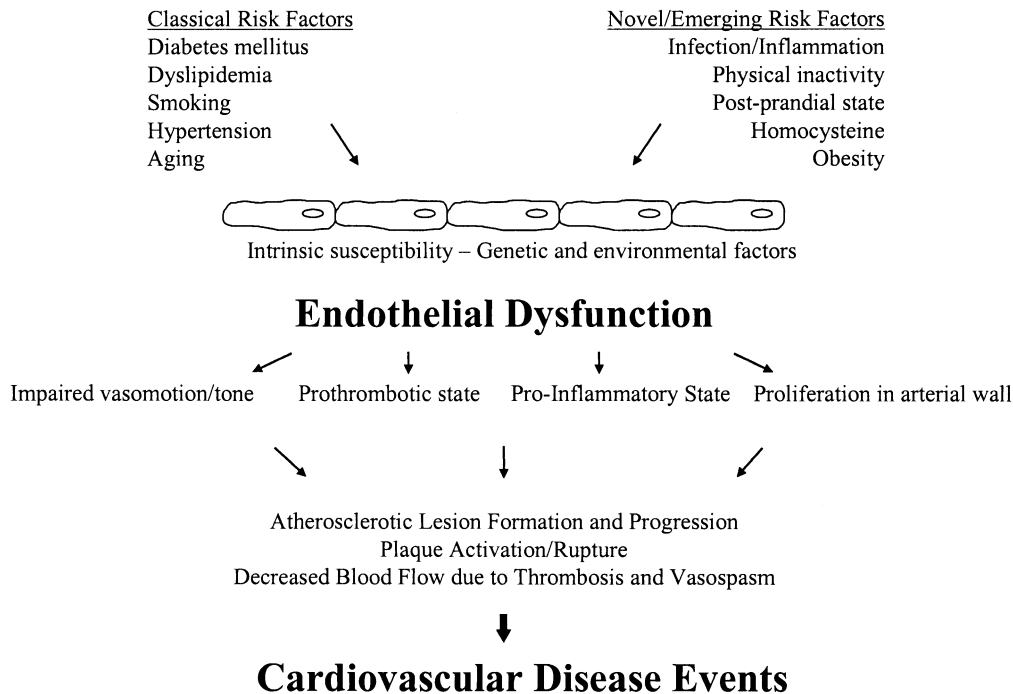


Figure 1. The role of endothelial dysfunction in the pathogenesis of cardiovascular disease events. Cardiovascular disease risk factors adversely affect a diverse range of endothelial homeostatic functions and contribute mechanically to the development, progression, and clinical expression of atherosclerosis. The response of the endothelium to the cumulative effects of risk factors may, in part, relate to intrinsic and environmental factors such as genetic polymorphisms, dietary factors, exercise, and other factors. Thus, endothelial function may serve as a barometer for cardiovascular risk.

parallel findings, one modest-sized study suggested that, within individual subjects, brachial artery FMD does not correlate with resistance vessel (microvascular) function as measured by infusion studies (26). Indeed, it is likely that there is differential regulation of vascular tone in conduit and resistance vessels, and that the different measures of vascular function may have relevance to different aspects of CVD.

Studies evaluating the prognostic value of endothelial dysfunction. Although case-control studies indicate an association between endothelial dysfunction and acute coronary syndromes (14), more convincing evidence for a pathogenic role is provided by studies demonstrating that endothelial dysfunction identifies patients at increased risk for future events. To date, 10 published studies have examined this issue (Table 3).

Three studies evaluated the prognostic value of endothelial dysfunction in the coronary circulation in patients with CAD (27–29). In each study, endothelial dysfunction predicted the occurrence of CVD events, such as cardiac death, myocardial infarction, unstable angina, ischemic stroke, and revascularization procedures, after controlling for known risk factors. The studies are limited because there was no prospective plan to obtain long-term follow-up at the time of enrollment and because the methods for studying endothelial function may have evolved over time. Nevertheless, these three studies involved a sizable number of patients and had consistent results. The study by Halcox *et al.* (29) is particularly convincing because of the larger sample size and

because the combined end point did not involve revascularization procedures, which, unlike spontaneous cardiovascular events, are more likely to be influenced by non-biological factors. In these studies, it is interesting that future events were poorly predicted by the angiographic severity of disease.

Two additional studies involved patients with CAD, but examined endothelial dysfunction in the brachial rather than coronary circulation. Heitzer *et al.* (30) observed that the forearm blood flow responses to intra-arterial acetylcholine was an independent predictor of cardiovascular events, further suggesting that the forearm circulation is a reasonable surrogate for the coronary circulation. These investigators also examined the degree to which a concomitant ascorbic acid infusion improved endothelial function. Patients with the largest improvement in endothelial function during ascorbic acid infusion had the highest risk, suggesting that increased oxidative stress may be a contributing mechanism for endothelial dysfunction and events. Neunteufl *et al.* (31) examined brachial artery FMD using ultrasound. Although limited by a relatively small sample size, retrospective design, and a heterogeneous mix of stable and unstable patients, this study also suggested that endothelial dysfunction in the brachial artery has prognostic value.

Gokce *et al.* (32) prospectively examined patients with atherosclerotic peripheral arterial disease awaiting non-emergent vascular surgery. Such patients are known to have a high incidence of recognized and undiagnosed CAD, and

Table 2. Advantages and Disadvantages of Methods to Quantify Endothelial Function in Humans

1. Intracoronary agonist infusion with quantitative coronary angiography
Advantages
Direct quantification of endothelial function in the vascular bed of interest
Allows for mapping dose-response relationships of endothelial agonists and antagonists
Allows for examination of basal endothelial function (with NOS antagonist infusion)
Disadvantages
Invasive
Expensive
Carries risks inherent with coronary artery catheterization (stroke, MI, infection, vascular injury)
2. Brachial artery catheterization with venous occlusive plethysmography
Advantages
More accessible circulation than coronary arteries
Allows for mapping dose-response relationships of endothelial agonists and antagonists
Allows for examination of basal endothelial function (with NOS antagonist infusion)
Disadvantages
Invasive
Risk of median nerve injury, infection, vascular injury
3. Vascular tonometry and measurements of vascular stiffness
Advantages
Noninvasive
Safer and faster than either invasive method
Lower operator dependence than brachial artery ultrasound
May reflect basal endothelial function
Disadvantages
Importantly influenced by structural aspects of the vasculature beyond the endothelium
4. Brachial artery ultrasound with FMD
Advantages
Noninvasive
Safer and faster than either invasive method
Reactivity correlates to endothelial dysfunction in coronary circulation
Flow is a physiological stimulus for vasodilation unlike agonists such as acetylcholine
Disadvantages
Poor resolution relative to arterial size
Variability in measurements
Highly operator-dependent

FMD = flow-mediated dilation; MI = myocardial infarction; NOS = nitric oxide synthase.

they have high short-term post-operative risk. Endothelial function was determined by brachial ultrasound before surgery, and patients were followed for 30 days after surgery. The study demonstrated that impaired FMD was a strong independent predictor of post-operative events. The post-operative state is associated with pain, fluid shifts, increased sympathetic nervous system activity, and inflammation, and, in this setting, endothelial dysfunction might increase the risk for plaque rupture or a mismatch between myocardial oxygen demand and supply. On longer term follow-up (mean of 1.2 years), impaired brachial artery FMD remained an independent predictor of events, even after the patients had recovered from the immediate stress of surgery (33). Notably, the study demonstrated that this non-

invasive method for studying endothelial function had high sensitivity and negative predictive value, suggesting that it might have utility as a screening test to identify low-risk patients who might undergo surgery without further evaluation.

In addition to studies that examined patients with established atherosclerosis, several studies have examined the prognostic value of endothelial function in patients with risk factors, but no known atherosclerosis. Two of these studies were done in the brachial artery (34,35). Perticone et al. (34) examined the forearm blood flow responses to acetylcholine in untreated male and female patients with hypertension, and observed that endothelial dysfunction identified patients at risk. Modena et al. (35) examined brachial artery FMD in post-menopausal women with newly diagnosed hypertension. Patients had increased risk over the next five years when endothelial dysfunction was not reversed by six months of antihypertensive therapy. Although treatment was not standardized, the type of antihypertensive therapy or the degree of blood pressure (BP) lowering did not explain the difference in prognosis. Importantly, these two studies raise the possibility that endothelial function could be used as a screening test for the primary prevention of CVD and as a guide to therapy.

Studies in patients with angiographically normal coronary arteries provide further evidence that endothelial dysfunction precedes and portends the development of atherosclerosis. Halcox et al. (29) found both epicardial and microvascular endothelial dysfunction predicted future cardiovascular events independently of the angiographic presence of CAD at the time of enrollment. Recently, Schindler et al. (36) reported that a coronary vasoconstrictor response to the cold pressor test, which reflects, in part, endothelial dysfunction, independently predicts future cardiovascular events in patients with normal coronary angiograms and elevated C-reactive protein levels.

Overall, the 10 studies examining the prognostic value of endothelial vasomotor function involved 1,920 patients with atherosclerosis or hypertension and 333 patients with events. These studies strongly and consistently demonstrate that endothelial dysfunction identifies patients who have increased risk for CVD events in the short and long term. Importantly, endothelial vasomotor dysfunction appears to be a systemic process that can be identified in vascular beds remote from the coronary and cerebral circulations where events occur.

In addition, vasomotor dysfunction, circulating blood markers of endothelial dysfunction, also have prognostic value. In patients without known CVD, elevated levels of soluble intercellular adhesion molecule (ICAM) (37), and tissue plasminogen activator (9), are independent predictors of future cardiovascular events. In patients with known coronary disease, soluble ICAM (38), von Willebrand factor (39), tissue plasminogen activator (39), plasminogen activator inhibitor (40), and endothelin (41) all have prognostic value. As mentioned previously, markers of systemic inflam-

Table 3. Studies Evaluating the Predictive Value of Endothelial Dysfunction

Lead Author	Design/ Mean Follow-Up	Patient Population	Vascular Bed	Marker of Endothelial Function	End Points Examined	Findings
Al Suwaidi (27)	Retrospective/28 months	157 patients with mild CAD	Coronary	Acetylcholine response	Cardiac death, MI, CHF, CABG, or PCI	6 patients with event. Acetylcholine response independent predictor of events.
Schachinger (28)	Retrospective/7.7 years	147 patients with CAD	Coronary	Acetylcholine, cold pressor test, FMD, NTG	MI, UA, ischemic stroke, CABG, PTCA, peripheral bypass	28 patients with event. Vasomotor function independent predictor of events.
Neunteufl (31)	Retrospective/5 years	73 patients with CAD	Brachial	FMD	Death, MI, PTCA, or CABG	27 patients with event. FMD <10% predictive of events. Effect lost when controlling for extent of CAD.
Heitzer (30)	Prospective/4.5 years	281 patients with CAD	Brachial	Forearm blood flow response to acetylcholine	CVD death, stroke, MI, CABG, PTCA, peripheral bypass	91 patients with event. Acetylcholine response independent predictor of events.
Perticone (34)	Prospective/32 months	225 patients with hypertension	Brachial	Forearm blood flow response to acetylcholine	CVD death, MI, stroke, TIA, UA, CABG, PTCA, PVD	29 subjects with event. Acetylcholine response predictive of events.
Gokce (32)	Prospective/30 days	187 patients undergoing vascular surgery	Brachial	FMD	CVD death, MI, UA, stroke	45 patients with event. FMD independent predictor of events.
Modena (35)	Prospective/67 months	400 hypertensive post-menopausal women	Brachial	FMD	Hospitalization for CVD event (not otherwise specified)	47 patients with event. Failure to improve FMD with 6 months of antihypertensive therapy independent predictor of events.
Halcox (29)	Retrospective/46 months	308 patients referred for cardiac catheterization	Coronary	Acetylcholine response	CVD death, MI, ischemic stroke, UA	35 subjects with event. Acetylcholine response independent predictor of events.
Schindler (36)	Prospective/45 months	130 patients with normal coronary angiograms	Coronary	Cold presser test	CVD death, UA, MI, PTCA, CABG, stroke, peripheral bypass	26 patients with event. Cold presser test response independent predictor of events.
Gokce (33)	Prospective/1.2 years	199 patients undergoing vascular surgery	Brachial	FMD	CVD, death, MI, UA, stroke	35 patients with events. FMD independent predictor of long-term events.

CABG = coronary artery bypass graft surgery; CAD = coronary artery disease; CHF = congestive heart failure; CVD = cardiovascular disease; FMD = flow-mediated dilation; MI = myocardial infarction; NTG = nitroglycerin-mediated dilation; PCI = percutaneous coronary intervention (e.g., angioplasty or stent); PTCA = percutaneous transluminal coronary angioplasty; PVD = peripheral vascular disease; TIA = transient ischemic attack; UA = unstable angina.

Table 4. Effect of Interventions on Endothelial Function and CVD

Intervention	Effect on Endothelial Function	Effect on CVD Events
Lipid-lowering therapy	+	+
Smoking cessation	+	+
Exercise	+	+
ACE inhibitors	+	+
Angiotensin receptor blockers	+	+
N-3 fatty acids	+	+
Glycemic control in diabetes mellitus	+	+
Hormone replacement therapy	±	—
Vitamin E	±	—
Combination antioxidants	—	—
L-arginine	+	?
Dietary flavonoids	+	?
Vitamin C	+	?
Folate	+	?
Tetrahydrobiopterin	+	?
Specific metal ion chelation therapy	+	?
Protein kinase C inhibition	+	?
Cyclooxygenase-2 inhibition	+	?
Thromboxane A ₂ inhibition	+	?
Troglitazone treatment in diabetes	+	?
Xanthine oxidase inhibition	+	?
Tumor necrosis factor inhibition	+	?

+ = weight of evidence indicates an improvement; — = weight of evidence indicates no effect or worsening; ? = there are insufficient data at the present time.
ACE = angiotensin converting enzyme; CVD = cardiovascular disease.

mation, including increased levels of C-reactive protein (7), are also associated with endothelial dysfunction in human subjects (8,42,43). Overall, these studies illustrate that identifying endothelial phenotype using systemic markers has prognostic value. It remains unknown which individual marker or combination of markers will prove most useful.

Interventions to reverse endothelial dysfunction. An important corollary to the hypothesis that endothelial dysfunction contributes to the pathogenesis of CVD is the idea that reversing endothelial dysfunction will reduce risk. Although this corollary has not been tested directly, numerous studies have evaluated lifestyle and pharmacologic interventions to improve endothelial function, and many of these same interventions are known to limit cardiovascular risk. The effects of some of these treatments on endothelial function and CVD risk are summarized in Table 4.

Lifestyle modification. Exercise is an important lifestyle factor that reduces cardiovascular risk (44), and exercise has been repeatedly shown to improve endothelial vasomotor function in healthy subjects (45,46) and in disease states including hypertension (47), congestive heart failure (48), and CAD (49). These effects appear to be mediated in large part by increased NO bioavailability (50) and may be greatest in vascular beds exposed to repetitive increases in blood flow during exercise (51), which includes the coronary circulation for all types of exercise.

In contrast, a sedentary lifestyle is linked to obesity and is associated with endothelial dysfunction, increased oxidative stress, and elevated systemic markers of inflammation. In

obese women, a yearlong program of low fat diet and exercise reduced plasma levels of tumor necrosis factor-alpha, interleukin-6, soluble ICAM-1, and soluble vascular cell adhesion molecule-1 (VCAM-1) (52). In that study, weight loss improved “endothelial function” as reflected by the degree of BP reduction after infusion of L-arginine. No published study has examined the effects of weight loss on endothelium-dependent vasodilation. Minimizing other traditional risk factors for CVD also improves endothelial function. For example, BP reduction (35), drug therapy to increase insulin sensitivity in diabetics (53), and smoking cessation (54) have been associated with improved endothelial function.

Dietary modifications. Diets low in fat and high in fruits and vegetables have been recommended by the American Heart Association to decrease cardiovascular risk (55). A portion of the benefit could result from increased intake of flavonoids, which may improve endothelial function. For example, endothelial dysfunction is reversed after intake of flavonoid-containing beverages including tea (56) grape juice (57), and de-alcoholized red wine (58).

Conversely, poor dietary habits may worsen endothelial function. Several studies suggest that a high-fat meal will induce acute impairment of FMD (59), although a portion of this effect may relate to other non-endothelium-dependent systemic effects on the vasculature (60). The type of fat consumed may also be important (61), as a diet high in n-3 fatty acids (i.e., fish oil) may improve endothelium-dependent vasodilation (62).

Antioxidant therapy. Oxidative stress is a central cause of endothelial dysfunction in atherosclerosis (63), and there has been great interest in the effects of antioxidant therapy. Regarding lipid-soluble antioxidants, probucol combined with lovastatin improved coronary endothelial function in patients with CAD (64). However, the data for vitamin E are quite mixed (reviewed by Duffy et al. [65]). Vitamin E has been shown to improve endothelial function in patients with multiple risk factors, particularly cigarette smoking (66). However, a number of other studies have failed to show a benefit (67–70). These latter results may be consistent with the recently published Heart Outcomes Prevention Evaluation (HOPE) study (71), which failed to demonstrate any effect of vitamin E on CVD events in a large-scale randomized trial.

Regarding water-soluble antioxidants, vitamin C administration consistently improves endothelium-dependent vasodilation in patients with CVD (65). Some epidemiologic studies suggest that individuals with low plasma concentrations (72) or low dietary intake (73) of ascorbic acid have increased cardiovascular risk. However, no randomized clinical trial has addressed the benefits of ascorbic acid treatment in a patient population with evidence of inadequate ascorbic acid intake or unsaturated tissue stores.

Studies of combinations of antioxidants, typically vitamin C, vitamin E, and beta-carotene, have provided disappointing results. Two studies failed to demonstrate a beneficial

effect of this combination on endothelium-dependent vasodilation (74,75). The recent Heart Protection Study examined such a combination in 20,536 individuals with CAD, diabetes, or peripheral vascular disease and demonstrated no benefit on cardiovascular events (76).

Despite the strong evidence that oxidative stress contributes to atherogenesis (77) and endothelial dysfunction (63), there are a number of possible reasons why antioxidant treatment has failed to show a benefit. For example, the studied antioxidants may have insufficient activity against the, as yet, undefined oxidants most relevant to CVD and endothelial dysfunction. The background antioxidant status of participants may have obscured any beneficial effect. Finally, it is possible that an antioxidant strategy designed to act on the sources of oxidant stress may be more effective than treatment with agents that act on selected “downstream” consequences, as has been suggested by Münzel and Keaney (78).

Lipid-lowering therapy. There is strong and consistent evidence that reduction of plasma low-density lipoprotein improves endothelial function. This benefit has been observed when low-density lipoprotein is lowered by non-pharmacologic means such as diet in animals (79), and with bile acid resins and plasma apheresis in humans (80,81). Treatment with HMG CoA reductase inhibitors (statins) has been consistently shown to reduce cardiovascular risk (82) and reverse endothelial dysfunction (64,80,83–86). Although two studies have failed to demonstrate a benefit on coronary endothelial function, these studies involved short-term treatment of patients with relatively low baseline cholesterol levels and had methodological problems including limited statistical power and improved endothelial function in the placebo group (87,88). While statins have been shown to induce regression of atherosclerotic plaques, the available data strongly suggest that the interrelated effects of statins on the endothelium, inflammation, and plaque composition are more important than lesion regression in regard to the observed reduction in cardiovascular risk (12).

While reduction of serum cholesterol is likely the major mechanism by which statins improve endothelial function, *in vitro* studies suggest that pleiotropic effects of statins may also be relevant. In addition to reducing cholesterol levels, HMG CoA reductase inhibition reduces cellular concentrations of important and biologically active intermediates that influence endothelial phenotype. By this mechanism, statins have been shown to directly enhance expression, phosphorylation state, and activity of the endothelial isoform of NO synthase (89,90). Moreover, C-reactive protein reduces NO synthase expression (91), suggesting that statins may specifically protect against the adverse effects of inflammation on the vasculature. It remains uncertain whether the pleiotropic effects of statins are relevant at the concentrations of statins achievable in patients.

Angiotensin-converting enzyme inhibition and angiotensin-II receptor blockade. Large-scale outcome trials (92) have clearly demonstrated that angiotensin-converting enzyme (ACE) inhibitors reduce CVD events in patients with CAD and diabetes, independent of BP reduction. Similarly, ACE inhibitors also improve endothelial function (93–96). Angiotensin converting enzyme inhibitors may affect endothelium-derived NO by several mechanisms. For example, angiotensin-II increases nicotinamide adenine dinucleotide phosphate (reduced) oxidase activity (97) leading to increased production of reactive oxygen species and “inactivation” of NO. Furthermore, ACE inhibitors inhibit the breakdown of bradykinin, a substance that stimulates NO production. Indeed, investigators have proposed that the balance between angiotensin II and NO is one of the major determinants of endothelial and vascular phenotype (98). The importance of angiotensin-II is further supported by the observation that angiotensin receptor blockers also appear to improve endothelial function and reduce endothelial markers of inflammation and oxidative stress (99,100).

Hormone replacement therapy. There has been extensive study of hormone replacement therapy (HRT) and endothelial function (101). Studies in post-menopausal women have repeatedly shown that estrogen replacement improves endothelium-dependent dilation and reduces systemic plasminogen activator inhibitor-1 levels (102). Combination therapy with a progesterone preparation blunts the benefits of estrogen in some, but not all studies (103,104). The issue of whether estrogen treatment has a beneficial effect on endothelial function in patients with established CVD has been less well studied, but a large cross-sectional study suggests that the beneficial effects are less than those observed in younger women without CVD (105). Despite the apparent beneficial effects on endothelial function, outcome studies have failed to show a beneficial effect of HRT (combination of estrogen and progesterone) for primary (106) or secondary (107) prevention of CVD events. Indeed, reduction in cardiovascular risk is no longer an accepted indication for HRT.

The explanations for the apparently disparate results remain uncertain. However, estrogen and progesterone have complex cellular effects, and it is possible that adverse effects, including pro-thrombotic effects, outweigh the benefits of improved endothelial function. Furthermore, it is unclear whether benefits of estrogen might have been confounded by concurrent progesterone therapy. Nevertheless, these results suggest that not every therapy that improves endothelial function translates directly into a reduction in cardiovascular risk.

Newer interventions. Finally, a number of newer therapies have been shown to improve endothelial function in human subjects, and a partial list is provided in Table 4. For example, L-arginine, which is the precursor for NO synthesis, has been administered in high doses to human subjects and has been shown in some studies to improve

Table 5. Clinical Utility of Endothelial Dysfunction

Current uses
Identification of novel risk factors for CVD
Investigation of mechanisms of atherosclerosis and vascular dysfunction
Surrogate marker of cardiovascular risk for intervention studies involving groups of patients
Potential future uses
Screening individuals for future cardiovascular risk
Evaluating CVD patients for lifestyle, pharmacologic, and/or mechanical intervention
Preoperative evaluation
Monitoring response to primary and secondary prevention therapies/strategies

CVD = cardiovascular disease.

endothelium-dependent dilation (108,109). Other examples include tetrahydrobiopterin, an essential co-factor for endothelial NO synthase (110), protein kinase C inhibition (111), iron chelation (112), and cyclooxygenase-2 inhibition (113). It is likely that many additional therapies will emerge as the pathophysiologic mechanisms of endothelial dysfunction in specific disease states are elucidated.

Clinical utility of studying endothelial function. In summary, the reviewed studies suggest that: 1) the endothelium plays a central role in vascular homeostasis and the pathogenesis of CVD; 2) endothelial vasomotor function can readily be measured in the coronary and peripheral circulations and that systemic markers of endothelial phenotype can be measured in blood; 3) endothelial vasomotor dysfunction detected in the coronary or peripheral circulation has prognostic value; and 4) many, but not all, interventions that reverse endothelial dysfunction also reduce cardiovascular risk. The question at hand is how these results can be used from a public health and/or clinical perspective (Table 5).

The available evidence suggests that endothelial function reflects the integrated effects of risk factors on the vasculature and that the development of endothelial dysfunction is an early event in the atherogenic process. There are strong and consistent relationships between mechanistically diverse risk factors and endothelial dysfunction. Furthermore, endothelial dysfunction identifies individuals at risk, before the development of clinically apparent CVD. These observations suggest that study of endothelial dysfunction has utility for the identification of novel risk factors for CVD. The finding that a potential risk factor is associated with endothelial dysfunction in carefully controlled cross-sectional studies would strongly suggest that this factor is associated with the development of CVD. Further evidence would be provided by studies showing the reversal of endothelial dysfunction by a specific intervention also reduces the cardiovascular risk associated with the risk factor. Often such studies are performed in the context of supportive epidemiologic outcome studies and mechanistic basic studies suggesting a causal relationship between the risk factor and atherosclerosis. Recent examples of the utility of endothelial function in regard to novel risk factors include obesity (6) and certain systemic infections (8).

Another current role for study of endothelial dysfunction is evaluation of interventions to reduce CVD risk. There is great interest in identifying "surrogate markers" of risk that can be used as an end point to evaluate a potential intervention before undertaking a longer term and considerably more expensive study that involves CVD events as the end point. Given the prognostic value of endothelial dysfunction and the strong correlation between improved endothelial dysfunction and reduced cardiovascular risk (Table 4), it is reasonable to consider endothelial dysfunction for this purpose. The possibility of using endothelial function to screen patients for evidence of high cardiovascular risk is further supported by high sensitivity and negative predictive values (>90%, respectively) (32). Again, studies evaluating the utility of endothelial function as a screening test must be evaluated in the context of other available epidemiologic, clinical, and experimental data. As is the case for HRT, potential confounding effects of the intervention must be considered.

A number of other modalities have been considered potential surrogate end points for CVD, including carotid-intimal thickness measured by ultrasound and coronary calcification assessed by computed tomography or magnetic resonance imaging scan. These modalities largely provide a measure of the presence and extent of fixed atherosclerosis. Studies of endothelial function may prove advantageous because they provide insight into vascular function, which appears to be more relevant to the pathogenesis of plaque rupture and the ensuing thrombosis that underlies cardiovascular events. Measurement of serum markers of inflammation (e.g., C-reactive protein) is another promising approach to this issue, but may not reflect the susceptibility of the vasculature to the adverse effects of systemic inflammation. It is possible that the state of the endothelium may reflect the degree to which the vasculature has been altered by inflammatory stimuli, and, thus, may provide additional prognostic information. Also unknown is the potential role of more specific serum markers of endothelial dysfunction such as plasminogen activator inhibitor-1, endothelin, and adhesion molecules (ICAM-1, VCAM-1). Direct comparative studies of the relative utility of available surrogates are lacking at the present time.

The available studies linking endothelial dysfunction to cardiovascular events (Table 3) raise the intriguing possibility that the technique could have utility for the management of individual patients. In regard to brachial artery FMD, studies of patients with hypertension and established coronary disease suggest that endothelial dysfunction identifies individuals who might benefit from more intensive treatment (31,35). Similarly, the prospective studies by Gokce et al. (32,33) might suggest that patients with peripheral arterial disease with preserved endothelial function are at low risk for perioperative and long-term events and might be managed differently than patients with poor function. The study by Modena et al. (35) raises the further possibility that persistent endothelial dysfunction during antihyperten-

sive therapy identifies high-risk individuals and that endothelial function might be used to monitor the effectiveness of risk reduction therapy. Thus, evaluation of endothelial function could be advantageous in prevention of both primary and secondary events. A paradigm shift from the current reactive, symptom-based, screening system looking for active disease to a non-invasive, relatively inexpensive, screening system based on vascular function would also be of benefit to both the general health of the population and the already overburdened and costly medical system.

Although highly appealing, there are insufficient data to support these possible applications for individual patients at the present time. Reproducible evaluation of endothelial function is limited to facilities with extensive experience in these techniques. The applicability of testing endothelial function on a population-wide basis is further diminished by the lack of large prospective trials evaluating its efficacy as a screening tool in the general population and by the lack of trials demonstrating that improving endothelial function decreases cardiovascular risk. Further studies are needed to confirm the available results and to carefully evaluate the sensitivity and specificity of the techniques relative to or in combination with other available measures of risk for individual patients. The recently initiated Multi-Ethnic Study of Atherosclerosis (MESA) will clarify some of these issues by simultaneously examining the predictive value of several measures of endothelial function and other subclinical markers of atherosclerosis (114). However, further studies are needed to demonstrate that clinical use of endothelial function can be used to guide risk reduction therapy.

Future directions. The available methods for studying endothelial function are currently useful for evaluating risk factors, mechanisms of CVD, and potential interventions in groups of patients. However, as outlined in Table 2, there are important limitations associated with each of these techniques. Development of improved or novel methodology to assess endothelial vasomotor function would be extremely useful. One approach would be to develop a means to obtain higher-resolution imaging of arterial diameter. Ideally, such imaging would be performed in the coronary circulation, although the available data indicate that peripheral arteries are reasonable surrogates. The most current non-invasive methodology requires off-line analysis, and another potential advance would be the development of continuous on-line measurement and reporting of vasomotor responses. At the present time, study of nitric-oxide-dependent responses requires imaging of blood vessels, measurement of changes in blood flow, or pulse wave analysis. Development of simpler indirect methods to assess endothelium-dependent responses may hold some promise for the future. For example, there is recent interest in a simple pulse amplitude tonometry method to measure FMD of small vessels in the finger (115–117). There also may be utility in further study of other manifestations of the pathologic endothelial phenotype, including pro-

thrombotic, vasoconstrictor, and pro-inflammatory factors that can be measured in blood. Most important for the future use of endothelial function in the care of patients is the need for a standardized approach that is supported by large-scale outcome studies.

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