ATVB in Focus
Noninvasive Assessment of Atherosclerosis: from Structure to Function

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Endothelial Dysfunction
A Marker of Atherosclerotic Risk

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

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

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

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

TABLE 1. Favorable and Atheroprotective Effects of the Healthy Endothelium
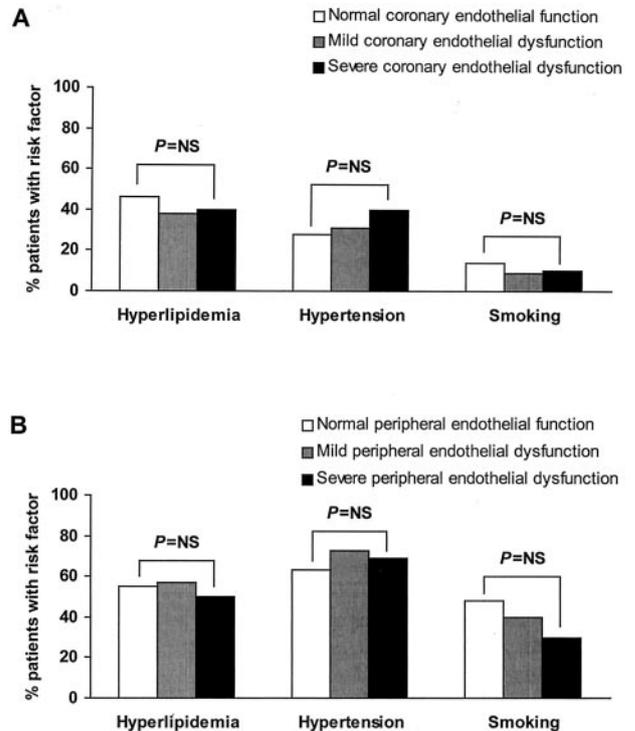
Promotion of vasodilation
Antioxidant effects
Antiinflammatory effects
Inhibition of leukocyte adhesion and migration
Inhibition of smooth muscle cell proliferation and migration
Inhibition of platelet aggregation and adhesion
Anticoagulant effects
Profibrinolytic effects

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

Endothelial Dysfunction Is a Systemic Disorder

Since the first description of endothelial dysfunction in atherosclerotic epicardial coronary arteries in 1986 by Ludmer and colleagues,[5] invasive assessment of coronary endothelial function by quantitative coronary angiography and coronary Doppler flow measurements, along with graded intracoronary infusions of endothelium-dependent vasodilators such as acetylcholine, may be considered the "gold standard" for endothelial function testing.[6] However, during the last decade, other less-invasive or noninvasive techniques for the assessment of endothelial function, including strain-gauge forearm plethysmography in conjunction with intra-arterial infusion of endothelium-dependent vasodilators, such as methacholine or acetylcholine, and high-resolution external vascular ultrasound to measure flow-mediated endothelium-dependent dilation (FMD) of the brachial artery during reactive hyperemia, have been developed. These techniques are based on the fact that endothelial dysfunction is not confined to the coronary arteries but rather represents a systemic disorder that also affects peripheral vascular beds, including both conduit arteries and small resistance vessels in the extremities.[7]

Given the systemic nature of endothelial dysfunction, the question arises as to whether peripheral vascular function parallels that of the coronary arteries and thus, may serve as a surrogate marker to identify individuals with coronary endothelial dysfunction. Indeed, 2 studies demonstrated a correlation between FMD of the brachial artery and epicardial coronary dilation in response to intracoronary infusion of acetylcholine or ATP, respectively, underscoring the systemic nature of endothelial function.[8,9] However, despite its widespread use as a research tool and the recent development of guidelines to standardize its use,[10] protocols for assessing FMD of the brachial artery still vary among different laboratories and are operator dependent.[4,10] This decreases the feasibility of this noninvasive technique as a valuable screening tool for endothelial dysfunction in clinical practice. Thus, there is growing interest in developing a noninvasive, reliable method to assess endothelial function.


Figure 1. Distribution of traditional cardiovascular risk factors in patients with normal endothelial function, mild endothelial dysfunction, or severe endothelial dysfunction of the coronary (A) or peripheral (B) vasculature. In these studies, prevalence of hypercholesterolemia, hypertension, and smoking status were similar in all groups irrespective of the status of endothelial function, indicating that the presence of known risk factors is not the only determinant of endothelial function. Data for A[16] and B[18] are from recent studies.

Endothelial Dysfunction and Risk Factors

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

Most if not all risk factors that are related to atherosclerosis and cardiovascular morbidity and mortality, including traditional and nontraditional risk factors, were also found to be associated with endothelial dysfunction. Many of these risk factors, including hyperlipidemia, hypertension, diabetes, and smoking are associated with overproduction of reactive oxygen species or increased oxidative stress.[11] By reacting with
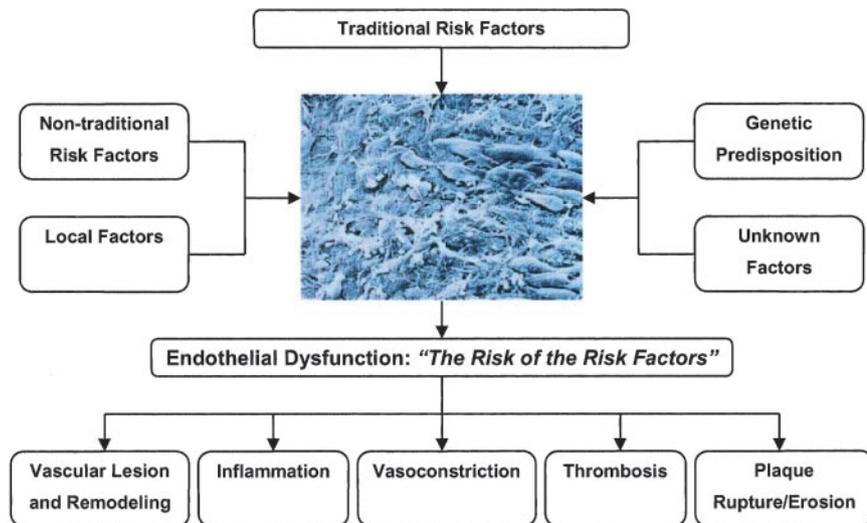
Figure 2. Endothelial dysfunction as the "risk of the risk factors." The endothelium represents a mechanical and biological barrier between the blood and the vascular wall. Traditional and nontraditional risk factors, local factors (eg, shear stress), genetic factors, and yet-unknown factors (protective or harmful) determine the status of endothelial function, which may be regarded as an integrated index of both the overall cardiovascular risk factor burden and the vasculoprotective factors in any given individual. The presence of endothelial dysfunction reflects a specific atherogenic vascular milieu, which is associated with perfusion abnormalities and cardiovascular events.

NO, reactive oxygen species may reduce vascular NO bioavailability and promote cellular damage.[12] Hence, increased oxidative stress is considered a major mechanism involved in the pathogenesis of endothelial dysfunction and may serve as a common pathogenic mechanism of the effect of risk factors on the endothelium.[11–13]

Some studies indicated that the risk to develop endothelial dysfunction increases with the number of risk factors present in an individual.[14,15] Also, assuming that the potential to alter endothelial function may vary between different risk factors, it may be speculated that certain clusters of risk factors, like those observed in patients with metabolic syndrome, may have a greater impact on endothelial function than other risk factor combinations. That the presence of established cardiovascular risk factors, however, is not the only determinant of endothelial function is pointed out by the observation that individuals with normal endothelial function and patients with various stages of endothelial dysfunction do not necessarily differ in their risk factor profiles (Figure 1).[16–18] Although local factors, in particular hemodynamic forces such as shear stress, have been recognized as important modulators of endothelial function[19] and therefore, may influence local endothelial homeostasis, these findings indicate a variable endothelial susceptibility of individual patients to cardiovascular risk factors and underscore the importance of other yet-unknown factors, including genetic predisposition, for both the prevention and promotion of endothelial dysfunction.

Taken together, the status of endothelial function represents an integrated index of both the overall cardiovascular risk factor burden and the sum of all vasculoprotective factors in any given individual.[20] Moreover, given its pivotal role in the atherogenic process, endothelial dysfunction may be regarded as the "ultimate risk of the risk factors" indicating the existence of a specific atherogenic vascular milieu (Figure 2).

Endothelial Dysfunction as a Clinical Syndrome

The presence of endothelial dysfunction can be regarded as a clinical syndrome that per se is associated with and predicts an increased rate of adverse cardiovascular events.[21–23]

Endothelial Dysfunction and Myocardial Ischemia

Coronary endothelial dysfunction, which is characterized by impaired NO bioavailability, may be associated with myocardial ischemia.[24] Both physical exercise[25,26] and mental stress,[27] 2 of the main triggers for an increase in myocardial demand, are associated with epicardial coronary endothelial dependant vasodilation. On the other hand, diverse studies indicated an association between the presence of coronary microvascular endothelial dysfunction and angina pectoris in patients with angiographically normal coronary arteries.[28–30] Zeiher and colleagues[31] demonstrated an association between impaired coronary endothelium-dependent vasodilation and exercise-induced myocardial perfusion defects in patients without hemodynamically relevant coronary artery disease (CAD). We provided evidence for a direct contribution of coronary endothelial dysfunction to myocardial ischemia by demonstrating the appearance of myocardial perfusion defects associated with intracoronary administration of acetylcholine in patients without obstructive CAD.[32] Hence, myocardial ischemia may result from endothelial dysfunction of either epicardial arteries or coronary microvessels.

Endothelial Dysfunction and Acute Coronary Syndromes

Acute complications play a crucial role in delineating the clinical expression of atherosclerotic disease. Endothelial dysfunction may play a fundamental role in the pathogenesis of acute coronary syndromes (ACSs), such as unstable angina and acute myocardial infarction.[33] Plaque destabilization, the detrimental process that predisposes the plaque to rupture, results from a complex interplay of inflammatory effects that involve cellular plaque components and various proinflammatory mediators.[34] Endothelial dysfunction is associated with increased oxidative stress, an important promotor of inflammatory processes.[35] NO may reduce endothelial expression of several inflammatory mediators and adhesion molecules that increase plaque vulnerability,[36,37] an effect that is mainly mediated by inhibition of the transcription factor nuclear factor-κB, a key regulator of various inflammatory proteins involved in atherosclerosis.[38,39] Thus, a dysfunctional endothelium may contribute to plaque destabilization owing to its reduced anti-inflammatory potential.

TABLE 2. Studies on the Prognostic Effect of Coronary and Peripheral Endothelial Dysfunction

Study	n	Population	Assessment of End Function	Mean Follow-Up, months	Cardiovascular Event Rate, %			End Dysfunction as an Independent Predictor of Events
					Normal End Function	Mild End Dysfunction	Severe End Dysfunction	
Al Suwaidi et al[16]	157	Pts without significant coronary stenoses	Coronary microvasculature	28	0	0	24	Not assessed
Schächinger et al[47]	147	Pts with various stages of CAD	Coronary macrovasculature	80				Yes
Halcox et al[17]	308	Pts with/without CAD	Coronary micro-/macrovasculature	46				Yes
Perticone et al[51]	225	Pts with untreated hypertension	Forearm microvasculature	32	5	11	23	Yes
Heitzer et al[52]	281	Pts with CAD	Forearm microvasculature	54				Yes
Gokce et al[18]	187	Pts undergoing vascular surgery	Brachial artery	1	8	32		Yes

End indicates endothelial; Pts, patients.

Precipitation of ACSs may also involve physical factors related to endothelial dysfunction. Given the increased vasoreactivity favoring local vasoconstriction in response to metabolic and sympathetic stimuli found in the area of culprit lesions in patients with unstable angina,[40] vasoconstriction associated with endothelial dysfunction may obviously represent a trigger for the physical disruption of coronary plaques.

Furthermore, a healthy endothelium is characterized by an antithrombotic milieu mediated by the secretion of various factors that exert antiaggregatory effects on platelets (NO and prostacyclin) or have anticoagulatory (heparin and protein C/S) or fibrinolytic (tissue plasminogen activator) properties.[41] Endothelial dysfunction is characterized by a reduction in the anticoagulatory potential of the endothelium and an increase in endothelial production of procoagulatory mediators (eg, tissue factor and plasminogen activator inhibitor),[41] resulting in a thrombogenic vascular environment. In addition, platelet-derived mediators, such as serotonin, induce vasoconstriction in the presence of a dysfunctional endothelium.[42] Moreover, vasoconstrictor response to these stimuli is increased by endothelin-1,[43] concentrations of which are elevated in the plasma of patients with early and advanced atherosclerosis[44,45] as well as in culprit lesions.[46]

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

Endothelial Dysfunction and Prognosis

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

First evidence of a prognostic effect of systemic endothelial dysfunction was suggested by data of the prospective "Men born in 1914" cohort, who underwent venous occlusion plethysmography of the calf between 1968 and 1970.[48] In this study, a low pulse-wave amplitude during reactive hyperemia was associated with a higher cardiac event rate and a higher all-cause mortality rate during 21 years of follow-up. Given that pulse-wave amplitude is negatively related to arterial stiffness and that endothelial dysfunction is associated with increased arterial stiffness[49,50] it can be speculated that patients with a low pulse-wave amplitude had peripheral endothelial dysfunction. Recently, 3 studies have shed more light on the relation between the presence of systemic endothelial dysfunction and an adverse outcome. Perticone and colleagues[51] followed up hypertensive patients who initially underwent endothelial function testing of the forearm vasculature by strain-gauge plethysmography in connection with intra-arterial infusion of graded doses of acetylcholine for an average of 31.5 months. During follow-up, cardiac, cerebrovascular, and peripheral vascular major adverse events occurred, whereby the risk to experience such an event depended on the magnitude of impairment of endothelial function of the forearm vasculature. In addition, multivariate analysis revealed the presence of severe peripheral endothelial dysfunction as an independent predictor of cardiovascular

events in this hypertensive population. In line with these findings, Heitzer and colleagues,[52] using the same methods, identified the presence of endothelial dysfunction in the forearm microvasculature as an independent predictor for an increased long-term cardiovascular risk in patients with documented CAD. Finally, Gokce and colleagues[18] measured FMD of the brachial artery in response to reactive hyperemia in 187 patients who subsequently underwent vascular surgery and prospectively followed up these patients for 30 days after surgery. During follow-up, a total of 45 patients experienced a postoperative cardiovascular event. Multivariate analysis identified impaired brachial FMD as an independent predictor of adverse short-term postoperative outcome.

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

Therapeutic Strategies to Improve Endothelial Function
Endothelial dysfunction is a reversible disorder, and strategies aimed at reducing cardiovascular risk factors, such as cholesterol lowering,[53] antihypertensive therapy,[54] smoking cessation,[55] estrogen replacement therapy in postmenopausal women,[56] supplementation with folic acid,[57] and physical exercise,[58] also translate into an improvement in endothelial health, further supporting the association between risk factors and endothelial dysfunction. Moreover, the observation that several pharmacological interventions that improve endothelial function are associated with a decrease in cardiovascular events independent of risk factor modification supports the concept that cardiovascular risk factors share a common pathway that leads to endothelial dysfunction, such as oxidative stress.

Although lowering of elevated cholesterol levels by itself is associated with an improvement in endothelial function independent of the therapeutic strategy used, treatment with statins was shown to be particularly effective for this purpose. Notably, a large body of experimental evidence indicates that statins may improve endothelial function by lipid-independent mechanisms, and recent reports suggest that similar statin effects may also exist in humans.[59] These "endothelium-protective" effects may be mediated by the statins' antioxidant and anti-inflammatory properties and their capability to restore vascular NO bioavailability.[59] Thus, it may be speculated that such properties are likely to

contribute to the favorable effect of these compounds on endothelial function and cardiovascular prognosis in both primary and secondary prevention.[60–64]

Angiotensin-converting enzyme inhibitors (ACEIs) increase NO bioavailability by decreasing the synthesis of angiotensin II and by enhancing serum levels of NO-releasing bradykinin via inhibition of its degradation. Moreover, ACEIs may also enhance the activity of endothelium-derived hyperpolarizing factor under certain conditions.[65] That short- and long-term ACEI administration may indeed translate into an improvement in both coronary and peripheral endothelial function was shown for patients with CAD and/or its risk factors,[66–69] although the magnitude of this effect may vary depending on the compound used, the presence of risk factors, and genetic variables.[70,71] In contrast to ACEIs, controversial results have been reported regarding the effect of angiotensin receptor antagonists, which specifically block the effects of angiotensin II on the angiotensin type 1 (AT_1) receptor, on endothelial function.[72,73] Given that increased oxidative stress plays a pivotal role in the pathogenesis of endothelial dysfunction,[11] administration of antioxidants would be expected to be a reasonable strategy to treat this disorder. Indeed, supplementation with antioxidants, such as glutathione,[74] *N*-acetylcysteine,[75] and, with a few exceptions,[76,77] vitamin C,[78] has been shown to reverse endothelial dysfunction in coronary and peripheral arteries of patients with cardiovascular risk factors and/or overt atherosclerosis. On the other hand, in contrast to in vitro studies and rather unexpectedly, current clinical data regarding the effect of vitamin E supplementation on endothelium-dependent vasoreactivity are inconsistent.[79]

The observation that interventions such as supplementation of the substrate for NO synthesis, L-arginine,[80] and calcium channel blockade with nifedipine[81] were shown to improve endothelial function without altering the risk factor profile further supports the concept that the endothelium serves as the common mediator of the detrimental effect of various cardiovascular risk factors.

Clinical Relevance of Therapeutic Improvement of Endothelial Function
Although various interventions were shown to be associated with improvement in endothelial function, little is currently known about the clinical and prognostic effect of therapeutic improvement in endothelial function. Cholesterol lowering with statins[82–84] and therapy with ACEIs[85] are also associated with a reduction of myocardial ischemia in patients with documented CAD. Moreover, the improvement in coronary endothelial function by L-arginine supplementation was associated with a reduction in anginal symptoms, suggesting a reduction in myocardial ischemia in patients with coronary endothelial dysfunction.[80] Taken together, these findings point out the clinical significance of therapeutic improvement in coronary endothelial function. In contrast, given the negative results of recent prospective long-term antioxidant supplementation trials[86,87] and the Heart and Estrogen/progestin Replacement Study (HERS),[88] direct proof for a causal relation between improvement in endothelial function and reduction in cardiovascular events has yet to be established.[89]

The reason for the lack of benefit of long-term antioxidant supplementation in the setting of primary and secondary prevention, however, is not clear. Given the strong relation between oxidative stress and endothelial function[11,12] and the observation that the level of oxidative stress may be an important determinant of clinical events,[52] it may be speculated that the potential of antioxidant supplementation to decrease cardiovascular events may be limited to a subgroup of individuals with elevated levels of endogenous oxidative stress and the very early stages of atherosclerotic disease. Furthermore, recent evidence suggests that certain antioxidants, including vitamin E, may be inappropriate to reduce oxidative stress in vivo or may even be pro-oxidant under certain conditions.[90] Thus, it is conceivable that combining different antioxidant compounds is essential for adequate reduction of oxidative stress and its sequelae.

Similarly disappointing were the negative results of the HERS trial, which failed to demonstrate a benefit of long-term hormone replacement therapy with conjugated equine estrogens plus medroxyprogesterone on coronary events in postmenopausal women with CAD. Although these results can at least partly be attributed to the coadministration of progesterone, which was shown to abrogate the positive effect of estrogens on endothelial function in some studies, the observation that administration of estrogens alone did not reduce progression of coronary atherosclerosis in women with established CAD indicates that other factors may have contributed to the negative findings of the HERS trial.[56,91] Most studies investigating the effect of estrogen replacement therapy (ERT) on vascular function assessed endothelial function in peripheral vessels.[56] Thus, a differential endothelial response to ERT between peripheral and coronary vessels cannot be ruled out. Also, it is possible that ERT restores only some endothelial properties, such as endothelium-dependent vasodilation, whereas a proinflammatory status may persist. This hypothesis is supported by the observation that ERT increases C-reactive protein levels.[92] Notably, in a substudy of the Cardiovascular Health Study, ERT improved brachial arterial endothelial function only in younger postmenopausal women without established atherosclerosis, whereas it had no effect in older women or in those with established atherosclerosis.[93] These results suggest that the beneficial effect of estrogen substitution on endothelial dysfunction and cardiovascular prognosis may be limited to younger postmenopausal women without established atherosclerosis.[93]

The overwhelming evidence from several large, clinical intervention trials in patients at risk for cardiovascular events, which showed a striking cardiovascular benefit with strategies that improve endothelial health, such as ACEIs[94] and statins,[60–64] support the notion that improvement in endothelial function may translate into a reduction of morbidity and mortality due to atherosclerotic disease. In summary, there is still a lack of direct proof that therapeutic improvement in endothelial function translates into lower cardiovascular morbidity and mortality, and further prospective trials are required to answer the question of whether enhancement of endothelial function should be considered a primary therapeutic end point.

Conclusions

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

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

Acknowledgments

References

1. Ross R. Atherosclerosis; an inflammatory disease. *N Engl J Med*. 1999; 340:115–126.
2. Kinlay S, Ganz P. Role of endothelial dysfunction in coronary artery disease and implications for therapy. *Am J Cardiol*. 1997;80:11-I–16-I.
3. Lerman A, Burnett JC Jr. Intact and altered endothelium in regulation of vasomotion. *Circulation*. 1992;86(suppl III):III-12–II-19.
4. Anderson TJ. Assessment and treatment of endothelial dysfunction in humans. *J Am Coll Cardiol*. 1999;34:631–638.
5. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med*. 1986;315:1046–1051.
6. Hasdai D, Lerman A. The assessment of endothelial function in the cardiac catheterization laboratory in patients with risk factors for atherosclerotic coronary artery disease. *Herz*. 1999;24:544–547.

7. Anderson TJ, Gerhard MD, Meredith IT, Charbonneau F, Delagrange D, Creager MA, Selwyn AP, Ganz P. Systemic nature of endothelial dysfunction in atherosclerosis. *Am J Cardiol*. 1995;75:71B–74B.

8. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, Yeung AC, Selwyn AP. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol*. 1995;26:1235–1241.

9. Takase B, Uehata A, Akima T, Nagai T, Nishioka T, Hamabe A, Satomura K, Ohsuzu F, Kurita A. Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease. *Am J Cardiol*. 1998;82:1535–1539.

10. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. *J Am Coll Cardiol*. 2002;39:257–265.

11. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res*. 2000;87:840–844.

12. Tomasian D, Keaney JF Jr, Vita JA. Antioxidants and the bioactivity of endothelium-derived nitric oxide. *Cardiovasc Res*. 2000;47:426–435.

13. Yura T, Fukunaga M, Khan R, Nassar GN, Badr KF, Montero A. Free-radical-generated F2-isoprostane stimulates cell proliferation and endothelin-1 expression on endothelial cells. *Kidney Int*. 1999;56:471–478.

14. Vita JA, Treasure CB, Nabel EG, McLenachan JM, Fish RD, Yeung AC, Vekshtein VI, Selwyn AP, Ganz P. Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. *Circulation*. 1990;81:491–497.

15. Celermajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *J Am Coll Cardiol*. 1994;24:1468–1474.

16. Al Suwaidi J, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation*. 2000;101:948–954.

17. Halcox JPJ, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA, Nour KRA, Quyyumi A. Prognostic value of coronary vascular endothelial dysfunction. *Circulation*. 2002;106:653–658.

18. Gokce N, Keaney JF Jr, Hunter LM, Watkins MT, Menzoian JO, Vita JA. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. *Circulation*. 2002;105:1567–1572.

19. Gimbrone MA Jr, Topper JN, Nagel T, Anderson KR, Garcia-Cardeña G. Endothelial dysfunction, hemodynamic forces, and atherogenesis. *Ann N Y Acad Sci*. 2000;902:230–239.

20. Vogel RA, Corretti MC. Estrogens, progestins, and heart disease: can endothelial function divine the benefit? *Circulation*. 1998;97:1223–1226.

21. Zeiher AM, Drexler H, Wollschläger H, Just H. Endothelial dysfunction of the coronary microvasculature is associated with impaired coronary blood flow regulation in patients with early atherosclerosis. *Circulation*. 1991;84:1984–1992.

22. McLenachan JM, Vita J, Fish DR, Treasure CB, Cox DA, Ganz P, Selwyn AP. Early evidence of endothelial vasodilator dysfunction at coronary branching points. *Circulation*. 1990;82:1169–1173.

23. Zeiher AM, Drexler H, Wollschläger H, Just H. Modulation of coronary vasomotor tone in humans: progressive endothelial dysfunction with different early stages of coronary atherosclerosis. *Circulation*. 1991;83:391–401.

24. Quyyumi AA, Dakak N, Andrews NP, Gilligan DM, Panza JA, Cannon RO III. Contribution of nitric oxide to metabolic coronary vasodilation in the human heart. *Circulation*. 1995;92:320–326.

25. Gage JE, Hess OM, Murakami T, Ritter M, Grimm J, Krayenbuehl HP. Vasoconstriction of stenotic coronary arteries during dynamic exercise in patients with classic angina pectoris: reversibility by nitroglycerin. *Circulation*. 1986;73:865–876.

26. Gordon JB, Ganz P, Nabel EG, Fish RD, Zebede J, Mudge GH, Alexander RW, Selwyn AP. Atherosclerosis influences the vasomotor response of epicardial coronary arteries to exercise. *J Clin Invest*. 1989;83:1946–1952.

27. Yeung AC, Vekshtein VI, Krantz DS, Vita JA, Ryan TJ Jr, Ganz P, Selwyn AP. The effect of atherosclerosis on the vasomotor response of coronary arteries to mental stress. *N Engl J Med*. 1991;325:1551–1556.

28. Motz W, Vogt M, Rabenau O, Scheler S, Luckhoff A, Strauer BE. Evidence of endothelial dysfunction in coronary resistance vessels in patients with angina pectoris and normal coronary angiograms. *Am J Cardiol*. 1991;68:996–1003.

29. Quyyumi AA, Cannon RO III, Panza JA, Diodati JG, Epstein SE. Endothelial dysfunction in patients with chest pain and normal coronary arteries. *Circulation*. 1992;86:1864–1871.

30. Egashira K, Inou T, Hirooka Y, Yamada A, Urabe Y, Takeshita A. Evidence of impaired endothelium-dependent coronary vasodilatation in patients with angina pectoris and normal coronary angiograms. *N Engl J Med*. 1993;328:1659–1664.

31. Zeiher AM, Krause T, Schächinger V, Minners J, Moser E. Impaired endothelium-dependent vasodilation of coronary resistance vessels is associated with exercise-induced myocardial ischemia. *Circulation*. 1995;91:2345–2352.

32. Hasdai D, Gibbons RJ, Holmes DR Jr, Higano ST, Lerman A. Coronary endothelial dysfunction in humans is associated with myocardial perfusion defects. *Circulation*. 1997;96:3390–3395.

33. Libby P. Current concepts of the pathogenesis of the acute coronary syndromes. *Circulation*. 2001;104:365–372.

34. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation*. 2002;105:1135–1143.

35. Napoli C, de Nigris F, Palinski W. Multiple role of reactive oxygen species in the arterial wall. *J Cell Biochem*. 2001;82:674–682.

36. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci U S A*. 1991;88:4651–4655.

37. De Catarina R, Libby P, Peng HB, Thannickal VJ, Rajavashisth TB, Gimbrone MA Jr, Shin WS, Liao JK. Nitric oxide decreases cytokine-induced endothelial activation: nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J Clin Invest*. 1995;96:60–68.

38. Barnes PJ, Karin M. Nuclear factor-κB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med*. 1997;336:1066–1071.

39. Peng HB, Libby P, Liao JK. Induction and stabilization of IκBα by nitric oxide mediates inhibition of NF-κB. *J Biol Chem*. 1995;270:14214–14219.

40. Bogaty P, Hackett D, Davies G, Maseri A. Vasoreactivity of the culprit lesion in unstable angina. *Circulation*. 1994;90:5–11.

41. McGorisk GM, Treasure CB. Endothelial dysfunction in coronary artery disease. *Curr Opin Cardiol*. 1996;11:341–350.

42. Golino P, Piscione F, Willerson JT, Cappelli-Bigazzi M, Focaccio A, Villari B, Indolfi C, Russolillo E, Condorelli M, Chiariello M. Divergent effects of serotonin on coronary-artery dimensions and blood flow in patients with coronary atherosclerosis and control patients. *N Engl J Med*. 1991;324:641–648.

43. Yang ZH, Richard V, von Segesser L, Bauer E, Stulz P, Turina M, Lüscher TF. Threshold concentrations of endothelin-1 potentiate contractions to norepinephrine and serotonine in human arteries: a new mechanism of vasospasm? *Circulation*. 1990;82:188–195.

44. Lerman A, Holmes DR Jr, Bell MR, Garratt KN, Nishimura RA, Burnett JC Jr. Endothelin in coronary endothelial dysfunction and early atherosclerosis in humans. *Circulation*. 1995;92:2426–2431.

45. Lerman A, Edwards BS, Hallett JW, Heublein DM, Sandberg SM, Burnett JC Jr. Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis. *N Engl J Med*. 1991;325:997–1001.

46. Zeiher AM, Goebel H, Schächinger V, Ihling C. Tissue endothelin-1 immunoreactivity in the active coronary atherosclerotic plaque: a clue to the mechanism of increased vasoreactivity of the culprit lesion in unstable angina. *Circulation*. 1995;91:941–947.

47. Schächinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation*. 2000;101:1899–1906.

48. Hedblad B, Ögren M, Janzon L, Isacsson SO, Lindell SE. Low pulse-wave amplitude during reactive leg hyperemia: an independent, early marker for ischaemic heart disease and death: results from the 21-year follow-up of the prospective cohort study 'Men born in 1914,' Malmö, Sweden. *J Intern Med*. 1994;236:161–168.

49. Kinlay S, Creager MA, Fukumoto M, Hikita H, Fang JC, Selwyn AP, Ganz P. Endothelium-derived nitric oxide regulates arterial elasticity in human arteries in vivo. *Hypertension*. 2001;38:1049–1053.

50. Wilkinson IB, Qasem A, McEniery CM, Webb DJ, Avolino AP, Cockcroft JR. Nitric oxide regulates local arterial distensibility in vivo. *Circulation*. 2002;105:213–217.

51. Perticone F, Ceravolo R, Pujia A, Ventura G, Iacopino S, Scozzafava A, Ferraro A, Chello M, Mastroroberto P, Verdecchia P, Schillaci G. Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation*. 2001;104:191–196.

52. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Münzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation*. 2001;104:2673–2678.

53. Vogel RA. Cholesterol lowering and endothelial function. *Am J Med*. 1999;107:479–487.

54. Taddei S, Virdis A, Ghiadoni L, Sudano I, Salvetti A. Effects of antihypertensive drugs on endothelial dysfunction: clinical implications. *Drugs*. 2002;62:265–284.

55. Celermajer DS, Sorensen KE, Georgakopoulos D, Bull C, Thomas O, Robinson J, Deanfield JE. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation*. 1993;88:2149–2155.

56. Vita JA, Keaney JF. Hormone replacement therapy and endothelial function: the exception that proves the rule? *Arterioscler Thromb Vasc Biol*. 2001;21:1867–1869.

57. Doshi SN, McDowell IFW, Moat SJ, Lang D, Newcombe RG, Kredan MB, Lewis MJ, Goodfellow J. Folate improves endothelial function in coronary artery disease: an effect mediated by reduction of intracellular superoxide? *Arterioscler Thromb Vasc Biol*. 2001;21:1196–1202.

58. Hambrecht R, Wolf A, Gielen S, Linke A, Hofer J, Erbs S, Schoene N, Schuler G. Effect of exercise on coronary endothelial function in patients with coronary artery disease. *N Engl J Med*. 2000;342:454–460.

59. Bonetti PO, Lerman LO, Napoli C, Lerman A. Statin effects beyond lipid lowering: are they clinically relevant? *Eur Heart J*. In press.

60. West of Scotland Coronary Prevention Study Group. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N Engl J Med*. 1995;333:1301–1307.

61. AFCAPS/TexCAPS Research Group. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. *JAMA*. 1998;279:1615–1622.

62. Scandinavian Simvastatin Survival Study Group. Randomized trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet*. 1994;344:1383–1389.

63. Cholesterol and Recurrent Events Trial Investigators. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. *N Engl J Med*. 1996;335:1001–1009.

64. The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. *N Engl J Med*. 1998;339:1349–1357.

65. Kansui Y, Fujii K, Goto K, Abe I, Iida M. Angiotensin II receptor antagonist improves age-related endothelial dysfunction. *J Hypertens*. 2002;20:439–446.

66. Mancini GBJ, Henry GC, Macaya C, O'Neill BJ, Pucillo AL, Carere RG, Wargovich TJ, Mudra H, Lüscher TF, Klibaner MI, Haber HE, Uprichard ACG, Pepine CJ, Pitt B. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease: the TREND study. *Circulation*. 1996;94:258–265.

67. Prasad A, Narayanan S, Husain S, Padder F, Waclawiw M, Epstein N, Quyyumi AA. Insertion-deletion polymorphism of the ACE gene modulates reversibility of endothelial dysfunction with ACE inhibition. *Circulation*. 2000;102:35–41.

68. O'Driscoll G, Green D, Rankin J, Stanton K, Taylor R. Improvement in endothelial function by angiotensin converting enzyme inhibition in insulin-dependent diabetes mellitus. *J Clin Invest*. 1997;100:678–684.

69. O'Driscoll G, Green D, Maiorana A, Stanton K, Colreavy F, Taylor R. Improvement in endothelial function by angiotensin-converting enzyme inhibition in non-insulin-dependent diabetes mellitus. *J Am Coll Cardiol*. 1999;33:1506–1511.

70. Anderson TJ, Elstein E, Haber H, Charbonneau F. Comparative study of ACE-inhibition, angiotensin II antagonism, and calcium channel blockade on flow-mediated vasodilation in patients with coronary disease (BANFF Study). *J Am Coll Cardiol*. 2000;35:60–66.

71. Mullen MJ, Clarkson P, Donald AE, Thomson H, Thorne SA, Powe AJ, Furuno T, Bull T, Deanfield JE. Effect of enalapril on endothelial function in young insulin-dependent diabetic patients: a randomized, double-blind study. *J Am Coll Cardiol*. 1998;31:1330–1335.

72. Prasad A, Halcox JPJ, Waclawiw MA, Quyyumi AA. Angiotensin type 1 receptor antagonism reverses abnormal coronary vasomotion in atherosclerosis. *J Am Coll Cardiol*. 2001;38:1089–1095.

73. Prasad A, Tupas-Habib T, Schenke WH, Mincemoyer R, Panza JA, Waclawiw MA, Ellahham S, Quyyumi AA. Acute and chronic angioten-

sin-1 receptor antagonism reverses endothelial dysfunction in atherosclerosis. *Circulation*. 2000;101:2349–2354.

74. Prasad A, Andrews NP, Padder FA, Husain M, Quyyumi AA. Glutathione reverses endothelial dysfunction and improves nitric oxide bioavailability. *J Am Coll Cardiol*. 1999;34:507–514.

75. Andrews NP, Prasad A, Quyyumi AA. N-Acetylcysteine improves coronary and peripheral vascular function. *J Am Coll Cardiol*. 2001;37:117–123.

76. Duffy SJ, Gokce N, Holbrook M, Hunter LM, Biegelsen ES, Huang A, Keaney JF, Vita JA. Effect of ascorbic acid treatment on conduit vessel endothelial dysfunction in patients with hypertension. *Am J Physiol Heart Circ Physiol*. 2001;280:H528–H534.

77. Raitakari OT, Adams MR, McCredie RJ, Griffiths KA, Stocker R, Celermajer DS. Oral vitamin C and endothelial function in smokers: short term improvement, but no sustained beneficial effect. *J Am Coll Cardiol*. 2000;35:1616–1621.

78. Carr A, Frei B. The role of natural antioxidants in preserving the biological activity of endothelium-derived nitric oxide. *Free Radic Biol Med*. 2000;28:1806–1814.

79. Visioli F. Effects of vitamin E on the endothelium: equivocal? α-tocopherol and endothelial dysfunction *Cardiovasc Res*. 2001;51:198–201.

80. Lerman A, Burnett JC Jr, Higano ST, McKinley LJ, Holmes DR Jr. Long-term L-arginine supplementation improves small-vessel coronary endothelial function in humans. *Circulation*. 1998;97:2123–2128.

81. Verhaar MC, Honing MLH, van Dam T, Zwart M, Koomans HA, Kastelein JJP, Rabelink TJ. Nifedipine improves endothelial function in hypercholesterolemia, independently of an effect on blood pressure or plasma lipids. *Cardiovasc Res*. 1999;42:752–760.

82. Gould KL, Martucci JP, Goldberg DI, Hess MJ, Edens RP, Latifi R, Dudrick SJ. Short-term cholesterol lowering decreases size and severity of perfusion abnormalities by positron emission tomography after dipyridamole in patients with coronary artery disease: a potential noninvasive marker of healing coronary endothelium. *Circulation*. 1994;89:1530–1538.

83. Van Boven AJ, Jukema JW, Zwinderman AH, Crijns HJGM, Lie KI, Bruschke AVG, on behalf of the REGRESS Study Group. Reduction of transient myocardial ischemia with pravastatin in addition to the conventional treatment in patients with angina pectoris. *Circulation*. 1996;94:1503–1505.

84. Andrews TC, Raby K, Barry J, Naimi CL, Allred E, Ganz P, Selwyn AP. Effect of cholesterol reduction on myocardial ischemia in patients with coronary disease. *Circulation*. 1997;95:324–328.

85. Prasad A, Mincemoyer R, Quyyumi AA. Anti-ischemic effects of angiotensin-converting enzyme inhibition in hypertension. *J Am Coll Cardiol*. 2001;38:1116–1122.

86. Collaborative Group of the Primary Prevention Project (PPP). Low-dose aspirin and vitamin E in people at cardiovascular risk: a randomised trial in general practice. *Lancet*. 2001;357:89–95.

87. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 6 high-risk individuals: a randomised placebo-controlled trial. *Lancet*. 2002;2053:23–33.

88. Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, Vittinghoff E. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. *JAMA*. 1998;280:605–613.

89. Cannon RO III. Cardiovascular benefit of cholesterol-lowering therapy: does improved endothelial vasodilator function matter? *Circulation*. 2000;102:820–822.

90. Landmesser U, Harrison DG. Oxidant stress as a marker for cardiovascular events: ox marks the spot. *Circulation*. 2001;104:2638–1640.

91. Herrington DM, Reboussin DM, Brosnihan KB, Sharp PC, Shumaker SA, Snyder TE, Furberg CD, Kowalchuk GJ, Stuckey TD, Rogers WJ, Givens DH, Waters D. Effects of estrogen replacement on the progression of coronary-artery atherosclerosis. *N Engl J Med*. 2000;343:522–529.

92. Koh KK, Schenke WH, Waclawiw MA, Csako G, Cannon RO III. Statin attenuates increase in C-reactive protein during estrogen replacement therapy in postmenopausal women. *Circulation*. 2002;105:1531–1533.

93. Herrington DM, Espeland MA, Crouse JR III, Robertson J, Riley WA, McBurnie MA, Burke GL. Estrogen replacement and brachial artery flow-mediated vasodilation in older women. *Arterioscler Thromb Vasc Biol*. 2001;21:1955–1961.

94. The Heart Outcomes Prevention Evaluation Study Investigators. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med*. 2000;342:145–153.