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The Assessment of Endothelial Function
From Research Into Clinical Practice

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The discovery of nitric oxide (NO) as a crucial endothelium-derived molecule for vascular relaxation and the recognition of the endothelium as more than a passive interface between blood and the vessel wall led to substantial progress in the field of vascular research.[1] Endothelial dysfunction is a pathological condition characterized mainly by an imbalance between substances with vasodilating, antimitogenic, and antithrombogenic properties (endothelium-derived relaxing factors)[2] and substances with vasoconstricting, prothrombotic, and proliferative characteristics (endothelium-derived contracting factors).[3] Among the most important vasodilator molecules, particularly in muscular arteries, is NO, which also inhibits other key events in the development of atherosclerosis such as platelet adhesion and aggregation, leukocyte adhesion and migration, and smooth muscle cell proliferation. Particularly in the microcirculation, prostacyclin and endothelium-derived hyperpolarization factors (an umbrella term for substances and signals hyperpolarizing vascular myocytes by opening voltage channels[4]) also play an important role.

Generally, loss of NO bioavailability indicates a broadly dysfunctional phenotype across many properties of the endothelium. Thus, the assessment of its vasodilator properties resulting from NO and other molecules may provide information on the integrity and function of the endothelium. Interestingly, most, if not all, cardiovascular risk factors are associated with endothelial dysfunction,[5] and risk factor modification leads to improvement in vascular function. Endothelial dysfunction has been detected in the coronary epicardial and resistance vasculature and in peripheral arteries, so endothelial dysfunction can be regarded as a systemic condition.[6] Importantly, the process of atherosclerosis begins early in life, and endothelial dysfunction contributes to atherogenesis and precedes the development of morphological vascular changes.[7]

Over the past 25 years, many methodological approaches have been developed to measure the (patho)physiological function of the endothelium in humans.[8] Although the ability to measure endothelial function has boosted clinical research in this field, its use as a clinical tool in daily practice is not established, nor has any method been recommended in clinical guidelines for planning primary or secondary prevention of vascular disease.

The aims of this review are to give a short overview of the most commonly used methods to measure endothelial function in humans, particularly noninvasive techniques (Table 1), and to summarize the clinical implications of endothelial dysfunction in the population and in individual patients. The possible future role of endothelial function measurement for individualized medicine is also considered.

Methods to Assess Vascular Function

The first demonstration of endothelial dysfunction in atherosclerotic coronary arteries using intracoronary infusion of acetylcholine and quantitative coronary angiography dates back to 1986 by Ludmer and colleagues.[9] Their seminal studies heralded an important shift in paradigm in the understanding of human atherosclerosis, which had previously been regarded as a purely structural disease. Their research drew attention to the functional manifestations of atherosclerosis such as exaggerated vasoconstriction as a consequence of poorly functioning endothelium. Later, less invasive techniques were developed using mainly the forearm circulation as a surrogate for coronary arteries.[6,10,11] All approaches have their advantages and disadvantages; most important, different vascular beds are examined (Figure). The basic principle, however, is similar: Healthy arteries such as

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Table 1. Advantages and Disadvantages of the Most Commonly Used Techniques to Assess Endothelial Function

Technique	Vascular Bed	Advantages	Disadvantages	Stimulus (Examples)
Coronary epicardial vasoreactivity (QCA)	Epicardial macrovascular Conduit arteries	Assessment directly in the coronary vascular bed Gold standard	Invasive Expensive Time intensive Limited to those undergoing coronary angiography Challenging for serial measurements	Ach Exercise Pacing CPT
Coronary microvascular function–Doppler wires	Coronary microvascular Resistance arteries	Assessment directly in the coronary microvasculature	Invasive Expensive Time intensive Limited to those undergoing coronary angiography Challenging for serial measurements	Ach Adenosine Papaverine
FMD	Brachial artery Conduit artery	Easy access Correlation with invasive epicardial vascular function Many outcome studies Inexpensive Possibility to assess other important parameters (flow, baseline arterial diameters, FMC)	Challenging to perform well Disparate protocols for performance and standardizations Need for standardization	Reactive Hyperemia
Venous occlusion plethysmography	Forearm vasculature Microvasculature	Easy access Vasoactive substances infused to generate a dose-response relationship Contralateral arm as a control	Invasive (cannulation of the brachial artery) Time consuming	Ach and other vasoactive substances
EndoPAT	Finger Microvasculature	Easy to access and perform Automated Low interobserver and intraobserver variability Correlation with invasive microvascular vascular function	Expense of disposable finger probes PAT signal influenced by variable non endothelial factors	Reactive hyperemia

QCA indicates quantitative coronary angiography; Ach, acetylcholine; CPT, cold pressor test; FMD, flow-mediated dilation; FMC, flow-mediated constriction; and PAT, peripheral arterial tonometry.

the coronary or brachial arteries dilate in response to reactive hyperemia (flow-mediated vasodilatation) or after pharmacological stimuli, including intra-arterial infusion of endothelium-dependent vasodilators such as acetylcholine, bradykinin, or serotonin, via release of NO and/or other endothelium-derived vasoactive substances.[2] In disease states, such endothelium-dependent dilatation is reduced or absent. However, regardless of which technique is applied, vascular responses are determined not only by the functional status of the vasculature at the place of measurement but also by the structural condition of the resistance arteries in the microvasculature. Furthermore, to differentiate endothelium-dependent from endothelium-independent responses, exogenous NO donators (eg, glycerol-trinitrate) or direct non–NO donators such as adenosine can be applied. Impaired endothelial-independent function is associated with structural vascular alterations and alterations in smooth muscles cells rather than changes in the endothelium.

Coronary Epicardial and Microvascular Function

To assess coronary endothelial function, a functional test is performed to measure epicardial and resistance vessel endothelial function. Although these methods are limited by the invasive nature, their advantage is to measure endothelial function directly in this clinically important vascular bed.

Epicardial Endothelial Function

To image vasomotor responses of epicardial coronary arteries, quantitative coronary angiography or intravascular ultrasound is used, and changes in vessel diameters and cross-sectional areas in response to endothelium-dependent interventions are documented. After acetylcholine infusion, vessels and segments with an intact endothelium vasodilate, whereas vessels and segments with dysfunctional or disrupted endothelium will respond with vasoconstriction as a result of direct activation of muscarinic receptors on vascular smooth muscle cells.[9] Similar induced functional changes in vascular reactivity have been demonstrated with salbutamol[12] and other substances (Table 2) and with more physiological

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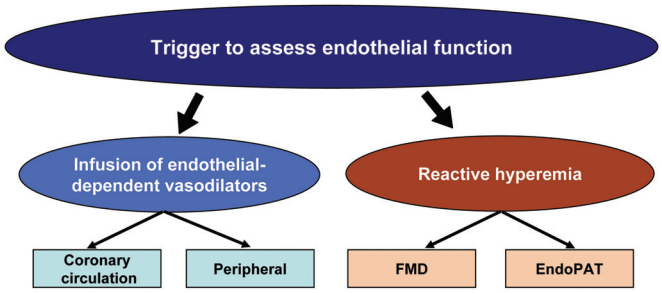

Non-Invasive	-	-	+	+
Predictive	++	++	++	+
Reversible	+	+	+	+
Control vessel	+ (control segments)	+	-	++
Not expensive	-	+	+	+/-
Low-risk	+/-	+	++	++
Operator independent	+/-	+/-	-	++
Easy to use	-	-	-	+

Figure. The principles of the most commonly used methods to assess endothelial function. FMD indicates flow-mediated vasodilatation.

interventions, eg, increased coronary blood flow. However, dose titration is more difficult. Physical measures of endothelium-dependent responses include exercise[13,14] or pacing-induced tachycardia as a surrogate for exercise[15,16] and induce an increase in coronary blood flow and thus shear stress on the coronary circulation, which leads to flow-mediated endothelium-dependent vasomotion of the epicardial vessels. Similar responses can be seen in response to mental stress.[17] The observation of endothelium-dependent flow-mediated dilatation in the coronary epicardial vessels and its impairment in atherosclerosis[18,19] provided the rationale to study similar responses in the peripheral vasculature later (see below). Another "physiological" test to assess epicardial vasoreactivity is the use of the cold pressor test in which the subject puts his or her hand into ice water. The activation of the sympathetic nervous system leads to release of NO and endothelium-derived hyperpolarizing factors via stimulation of endothelial α_2-adrenergic receptors and consequently vasodilation in healthy arteries.[20] However, in dysfunctional endothelium, α_1-adrenergic–mediated constriction of smooth muscle cells will dominate,[21] closely mirroring the responses to acetylcholine.[21,22]

Table 2. Pharmacological Triggers for the Assessment of Coronary Vascular Function

	Epicardial Vessels	Microcirculation
Endothelium-dependent vascular function	Acetylcholine	Acetylcholine
	Salbutamol	Salbutamol
	Serotonin	Bradykinin
	Substance P	
	Calcitonin gene-related peptide	
Endothelium-independent vascular function	Nitroglycerin	Adenosine
	Nitroprusside	Dipyridamole
	Papaverine	Nitroprusside
		Papaverine

Coronary Microvascular Function

Changes in coronary (or myocardial) blood flow can be used as a surrogate parameter for microvascular function.[23] Coronary flow reserve is the ratio of maximal coronary blood flow during maximal coronary hyperemia with provocative stimuli (such as adenosine infusion, pacing, or exercise) divided by the resting coronary blood flow. This maximal blood flow response (coronary flow reserve) is both endothelium- and non–endothelium-dependent, and a coronary flow reserve <2.0 is considered abnormal.[24] To measure endothelium-dependent microvascular function, the percent increase in coronary blood flow in response to endothelium-dependent vasodilators (commonly acetylcholine) infused at increasing concentrations is analyzed.

Other methods to estimate microvascular function have been introduced, eg, the measurement of the number of cineangiographic frames that it takes to fill a distal vessel with proximal injection of contrast. The corrected Thrombolysis in Myocardial Infarction frame count provides a semiquantitative assessment of epicardial coronary blood flow.[25] Taking the main disadvantage—the invasive nature of the above-mentioned tests—into account, noninvasive functional tests to assess the coronary microvasculature have been developed, among them positron emission tomography,[26] myocardial perfusion imaging,[27] blood oxygen level–dependent magnetic resonance imaging,[28] and echocardiography[29]; however, a detailed discussion of these tests is beyond the scope of this review.

Peripheral Techniques to Assess Endothelial Function

The aforementioned techniques to measure coronary epicardial vascular function and to assess the coronary microcirculation are very well suited for patients requiring a coronary angiogram for clinical indications. However, to assess vascular function and health in the asymptomatic patient, performing an invasive functional coronary angiogram is usually

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not appropriate. Therefore, noninvasive or less invasive surrogate techniques to assess macrovascular and microvascular endothelial function have been developed. Although they do not measure vascular function in the coronary circulation directly, they have been shown to correlate reasonably with its more invasive counterparts.[30–32] Whereas all these techniques assess the generalized function of the vasculature, one has to keep in mind that certain phenomena cannot be explained by systemic endothelial dysfunction; it is likely that local factors (eg, flow patterns) and local vascular dysregulation observed at branch points related to disturbed shear stresses also contribute to disease.[33–37]

Plethysmography of the Forearm Circulation

Although still limited by its semi-invasive nature (arterial puncture), this technique measures changes in forearm blood flow by venous plethysmography in both arms before and after infusion of vasoactive substances into a cannulated brachial artery.[10] The main advantage is that vasoactive molecules, hormones, or drugs (eg, acetylcholine or nitroglycerin) can be infused, thus quantifying endothelium-dependent and endothelium-independent vasodilation in a dose-dependent manner. The dosages required have limited systemic effects, allowing the contralateral limb to serve as an internal control. The results are expressed as the ratio of the changes in flow measured in both arms and are reproducible.[38] The response to acetylcholine is significantly reduced by intra-arterial infusion of N^G-monomethyl-L-arginine (but not by acetylsalicylic acid),[39] demonstrating a key role for NO. However, it has to be taken into account that, especially in patients with multiple risk factors, endothelium-derived hyperpolarization factors also play an essential role for resting microvascular tone[40] and for agonist-stimulated vasodilation.[41,42] The technique is well suited to measure differences in blood flow to various stimuli or inhibitors in a single patient. However, because of different initial arterial pressures, forearm blood flow, different sizes of the forearm, and other factors, comparisons between groups or serial studies in the same patient are of limited value.[43] Although pharmacologically induced vasodilation with this technique gives interesting insights into microvascular pathophysiology, the response not necessarily mimics microvascular vasodilation to transient ischemia or exercise.

Flow-Mediated Vasodilation of Brachial Artery

As a result of its noninvasive approach, flow-mediated vasodilatation of the arm arteries (FMD) has become the most widely used technique to measure endothelial function. The technique measures the ability of the arteries to respond with endothelial NO release during reactive hyperemia (flow mediated) after a 5-minute occlusion of the brachial artery with a blood pressure cuff. Celermajer and colleagues[11] were the first to measure this response in vivo by measuring the respective diameter changes of the brachial or radial artery by ultrasound, a response later demonstrated to be mainly NO dependent,[39,44,45] although other vasodilator pathways also may contribute.[46] Importantly, peripheral endothelial function as assessed by FMD correlates with coronary artery endothelial function.[30,32] However, although the principle of this technique seems simple, its application is technically challenging and requires extensive training and standardization (Table 3).[47–51] Study preparation, image acquisition and site selection, sphygmomanometer probe position, cuff occlusion time, accurate use of edge-detection software, and correct

Table 3. Technical Considerations in Flow-Mediated Dilation Measurements

Subject preparation
Fasting state (>6 h)
No smoking or any tobacco consumption at least 6 h before study
No exercise or food/beverages that contain alcohol or caffeine or are rich in polyphenols (cocoa, tea, fruit juices) for >12 h
No vitamins for at least 72 h
Vasoactive medications withheld on the morning of the study if possible with careful noting of the use and timing of any drugs
No exercise >12 h before test
Quiet, temperature-controlled room
In female patients, repetitive studies should be made at the same time of the menstrual cycle (ideally on days 1–7 of the menstrual cycle)
Rest for at least 10 min before measurements
Supine position
Arm resting comfortable with cradle support with the imaged artery at the heart level
Test should be performed at the same time of the day (especially if multiple tests are performed)
Sphygmomanometer probe position and cuff occlusion time
Placement of the cuff 1–2 cm distal to the elbow crease
Other sites are discouraged because proximal cuff positioning affects the magnitude of the peak vasodilatory response
Occlusion time, 5 min (shorter inflation attenuates FMD response)
Cuff inflation to at least 50 mm Hg above systolic pressure
Site selection
Brachial artery with a minimum diameter (usually >2 mm); small arteries are difficult to measure, and changes in absolute diameter correspond to big relative changes
If repetitive measurements are planned, site has to been replicated; anatomic landmarks should be used
Image acquisition
Longitudinal images obtained by high-resolution ultrasound (7.5–12 MHz)
A clear interface between the near and far arterial wall should be achieved
Diameter measurements are obtained in end diastole or averaged over the heart cycle
Stereotactic adjustable prop holding is essential to ensure image quality
Recording of the baseline diameter for at least 1 min
Simultaneous acquisition of pulse-wave Doppler velocity signals for quantification of shear stress (stimulus) if feasible; insonation angle should be <60°
Measurement
Automated edge detection should be used
Reported as maximal percentage change from baseline diameter (most reproducible)
Baseline diameter and absolute change reported also
Characterization of the hyperemic stimulus (ideally the flow-velocity time integral)

FMD indicates flow-mediated dilation. Sources of information: References 49 through 51.

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characterization of the FMD response are crucial, as recently outlined in detail in guidelines by Charakida et al,[49] Harris et al,[51] and Thijssen et al.[50] These publications are useful in that they draw attention to the need to standardize the different protocols; indeed, if efforts to standardize the technology are followed, reproducibility of FMD can be considerably improved.[52]

Recently, some new aspects of this technique have emerged and are under investigation. Although FMD measures conduit artery vascular function, the stimulus for FMD itself (reactive hyperemia flow and the induced shear stress on the endothelium) might be an important measure of peripheral microvascular function because reactive hyperemia is highly dependent on maximal forearm resistance.[53,54] Notably, both hyperemia-induced shear stress and velocity changes (measured by calculation of the velocity-time integral adjusted for heart rate) showed even stronger correlations with the presence of cardiovascular risk factors than FMD[55] and predict cardiovascular outcomes.[56,57]

Interestingly, some recent multicenter studies demonstrated that simple baseline brachial artery diameter readings correlate with clinical outcomes nearly as well as FMD itself.[58,59] One explanation for this intriguing finding might be that the larger the diameter of an artery is, the smaller the relative percent changes (FMD) are, and the shear stress generated seems to be lower in larger vessels.[60,61] Because shear stress is the stimulus for FMD, it seems reasonable to correct for the impact of shear stress on FMD. However, several methodological and physiological factors influence shear stress, and ratio normalizations of the dilatory response to shear rate have provided conflicting results.[50]

Recently, Gori et al[62] raised the issue of endothelial function in the resting (not hyperemic) state. Although FMD provides crucial information about the ability of the endothelium to respond to a specific stimulus (reactive hyperemia), it is not a measure of the resting production of vasoactive substances. In this respect, vasoconstriction of the brachial artery after inflation of a wrist cuff to suprasystolic pressure was first reported years ago[63] and is mediated mainly through vasoconstrictor substances such as endothelin.[64] This concept has garnered new attention, and the term low-flow–mediated constriction was introduced.[62,65,66] In principle, low-flow–mediated constriction detects the change in brachial artery diameter in response to a decrease in blood flow and shear stress after occlusion of the artery by a distally placed cuff. In a recent study, an association between traditional risk factors and impaired low-flow–mediated constriction and a relationship with the severity of coronary artery disease have been demonstrated.[67]

Taken together, the information obtained by functional vascular ultrasound is manifold. Future protocols not only should measure percent changes in arterial diameter in response to hyperemia but also should consider the above-mentioned measurements.

Finger Plethysmography

Measuring endothelial function with peripheral arterial tonometry has recently gained increased attention, and a proprietary device has been developed to measure observer-independent pulsatile arterial volume changes by finger plethysmography (EndoPAT; Itamar Medical).[68,69] With peripheral arterial tonometry, beat-to-beat plethysmographic recordings of the finger arterial pulse wave amplitude are captured with pneumatic probes. With the device, a counterpressure of 70 mm Hg on the digit is applied to avoid distal venous distention, thus inhibiting venous pooling and venoarteriolar reflex responses.[68] In principle, an increase in arterial blood volume in the finger tip causes an increase in pulsatile arterial column changes, thus increasing the measured signal. Similar to the assessment of endothelial function with the FMD technique, a pressure cuff is placed on the arm, and after baseline blood volume changes are obtained, the blood pressure cuff is inflated above systolic pressure and deflated after 5 minutes to induce reactive hyperemia on 1 arm. A main advantage of the system is that the contralateral arm serves as its internal control that can be used to correct for any systemic drift in vascular tone during the test, and an index between the 2 arms is calculated to adjust for any such drift. This index is a validated marker for endothelial function; however, augmentation of the pulse amplitude after reactive hyperemia is a complex response to ischemia. It reflects changes in flow and in digital microvessel dilatation and is only partly dependent on NO.[70] Validation studies demonstrated that impairment in peripheral finger endothelial function measured with EndoPAT is correlated with coronary microvascular function in patients with early atherosclerosis[31] and predicts cardiovascular events.[71] In 2 large cross-sectional studies (in >1900 patients in the Framingham cohort[72,73] and >5000 individuals in the Gutenberg Heart Study[74]), digital vascular dysfunction was associated with traditional and metabolic cardiovascular risk factors but not or only modestly with FMD, thus likely measuring different aspects of vascular biology (see below).

Clinical Implications of Endothelial Dysfunction in Populations and in the Individual

Is Endothelial Function a Marker for Cardiovascular Risk?

In the coronary arteries, impairment of endothelial function occurs early in the course of atherosclerosis in relation to systemic risk factors[75] and abnormal hemodynamic shear stresses.[37] The more systemic cardiovascular risk factors are present, the worse epicardial vascular function is.[75] Extensive literature documents that endothelial dysfunction is associated with almost every condition predisposing to atherosclerosis and cardiovascular disease.[76] There are many studies correlating endothelial dysfunction (conduit artery and microvasculature likewise) with cardiovascular risk. For example, endothelial dysfunction has been observed in patients with arterial hypertension,[10,77,78] normotensive subjects with a family history of hypertension,[79] smokers,[80,81] passive smokers,[82,83] patients with dyslipidemia,[85,86] ageing patients,[10] those with diabetes mellitus,[86–90] obese individuals[90] patients with hyperhomocysteinaemia,[91,92] individuals with low intracellular magnesium levels,[93] and patients with inflammatory or infectious diseases.[94–96] Importantly, the effects of cardio-

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vascular risk on the endothelium can be seen in children as early as 8 years of age.[97,98] Thus, endothelial dysfunction may represent the effect of these risk factors on vascular health.

The fact that endothelial dysfunction is a systemic condition[6] may explain why peripheral endothelial function (microvascular and macrovascular) correlates with endothelial function in the coronary arteries.[30–32] The pathophysiology behind the functional changes in impaired endothelial function also leads to structural changes of the vessel over time. In a cross-sectional study in healthy middle-aged men, there is no evident correlation between brachial FMD and the carotid intima-media thickness (IMT)[99]; however, in a similar population free of cardiovascular disease, FMD correlated with IMT progression over a 6-year follow-up. Interestingly, in this study, in contrast to FMD, Framingham risk was not correlated with IMT progression.[100] Similarly, FMD also predicted IMT progression in less healthy patients as demonstrated after 1 year in hypertensive, postmenopausal women.[101]

Taken together, there is good evidence that endothelial dysfunction is significantly associated with the burden of cardiovascular risk and can be considered a barometer of the total risk burden (the risk of the risk factors). However, transient endothelial function impairment, eg, by intercurrent acute illnesses,[98,102,103] after strenuous exercise,[104] or with certain foods,[105] has to be taken into account, posing a potential limitation for interpretation. Thus, it may be that endothelial function measurements should not rely on a single test but rather on the average of several tests.

Does Endothelial Function Provide Prognostic Information Beyond Commonly Used Risk Scores in Primary Prevention?

As outlined above, endothelial dysfunction is an important mechanism for cardiovascular risk; therefore, its association with prognosis is not surprising. In the clinical setting, however, it is relevant to ask whether endothelial dysfunction provides additional information beyond traditional risk score algorithms, defined, for example, by the Framingham, Prospective Cardiovascular Munster Study (PROCAM), or Systematic Coronary Risk Evaluation (SCORE) projects, or if it just reflects cardiovascular risk. Early invasive studies already demonstrated that endothelial dysfunction measured in the coronary vasculature is an important prognosticator for the incidence of further cardiovascular events even in patients without coronary artery disease.[106,107] However, in the setting of primary prevention, invasive measurements are not feasible, and most studies using peripheral endothelial function tests addressing this issue were limited by the small sample size, evaluation of a particular subset of patients, long follow-up periods required, or difficulties in correcting for the influence of preexisting cardiovascular risk factors.

Peripheral FMD is predictive of cardiovascular events beyond traditional risk factors in special subsets of patients such as after elective vascular surgery,[108] in postmenopausal women,[109] and in patients with chest pain.[110] Several (but not all) large-scale studies recognized the additional value of endothelial function in the primary prevention setting.

In 1 study of 435 and 1 study of 268 consecutive healthy subjects without heart disease and low clinical risk, brachial artery FMD independently predicted long-term adverse cardiovascular events in addition to traditional risk factor assessment.[111,112] In the Cardiovascular Health Study, the relationship between endothelial function (as measured by FMD) and subsequent cardiovascular events was measured in a cohort of >2700 apparently healthy subjects >72 years. Over a 5-year follow-up period, event-free survival was significantly higher in those patients with normal endothelial function, a relation that held true after adjustment for traditional risk factors.[58] Similarly, in the Multi-Ethnic Study of Atherosclerosis, a study in white, black, Hispanic, and Chinese subjects (>3000 persons), FMD again predicted future cardiovascular events, even after adjustment for the Framingham Risk Score. Furthermore, FMD in combination with the Framingham score helped to classify cardiovascular risk better than FMD or the Framingham score alone.[59] Similar to the above-mentioned study, in another large cohort (n>2000) of asymptomatic postmenopausal women, FMD provided additional prognostic information to the Framingham Risk Score.[113]

In these studies, the adjustments for traditional risk factors weakened the correlation of endothelial function and outcomes. This is not surprising because endothelial dysfunction is a key biological mechanism by which cardiovascular risk factors exert their propensity for atherosclerosis and adverse events. However, as seen in a large number of patients in the above-mentioned studies, endothelial function added further information, potentially suggesting that we have not yet found all individual risk factors and that in each individual subject, risk factors alone may not be linked predictably to clinical outcome. It also has to be taken into account that predictors found in multivariate analysis may be of only restricted clinical utility and provide only limited information for individual risk assessment.

In discussing whether endothelial dysfunction is able to predict future events beyond traditional risk scores, we have to take into account the vascular bed and the patient population in which the measurements were performed. In the Firefighters and Their Endothelium (FATE) study, for example, which included 1574 middle-aged apparently healthy men at low cardiovascular risk (Framingham Risk Score, 7.9%), FMD was not associated with future clinical events. One explanation for the neutral findings in terms of FMD might be that these firefighters were very physically active, with the consequence of adaptive vascular remodeling and thus lower FMD.[114] An interesting finding in the FATE study was that hyperemic velocity (a measure of microvascular function) in the brachial artery was significantly related to events in a multivariable analysis (also containing Framingham Risk Score).[57] Similar to the above-mentioned studies, the addition of hyperemic velocity (instead of FMD) to the Framingham Risk Score led to a risk-reclassification improvement. In another recent large (1016 persons) community-based cohort study, forearm microvascular endothelium function as assessed with acetylcholine infusion was associated with cardiovascular events in elderly patients, whereas FMD was not.[115] Again, adding microvascular endothelial dysfunction to the Framingham score improved risk

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discrimination. Similarly, microvascular endothelial dysfunction measured with EndoPAT was useful in predicting nonobstructive coronary atherosclerosis, which is not well predicted by the Framingham score,[116] and in independently predicting adverse cardiac events in 270 outpatients.[71] Thus, microvascular and macrovascular function may give important extra information, above and beyond traditional risk factors, in certain situations. If the above-mentioned studies are taken together, it seems that macrovascular endothelial function might be more important in patients with existing atherosclerosis but microvascular function might be more important in the younger subjects; in other words, microvascular function may be an earlier indicator of risk.

Despite data indicating a predictive value for future cardiovascular events, even after adjustment for known risk, endothelial function measurements are not yet recommended by guidelines for prevention by either the European (European Society of Cardiology)[117] or the more recent American (American Heart Association/American College of Cardiology)[118,119] guidelines (Class III indication) and received lower classification of recommendation than carotid IMT measurements and coronary calcium score. Reasons for this Class III indication were the lack of clear additional prognostic value of endothelial function and the poorly standardized noninvasive methodology (except EndoPAT). However, most of the above-mentioned FMD studies that demonstrate a clear addition of prognostic value are rather new and were published after the release of the guidelines. Despite the high sensitivity as a prognosticator for future events, specificity remains a concern. However, with technical modifications and more accurate analysis software, the variability of FMD measurements and thus the specificity can be shown to be further improved. If endothelial function is appreciated as a dynamic process (perhaps several measurements should be averaged) and if standardized protocols are followed, reproducible measurements can be achieved, especially if performed in high-volume experienced centers.[49] Furthermore, IMT and calcium score, although they can be performed in a more standardized manner and are less affected by transient abnormalities than endothelial function, give information about the vascular structure (more established disease) rather than function. Additionally, these measurements do not change rapidly with interventions, an invaluable advantage of endothelial function measurements.

Is There Any Role of Endothelial Function for Prognosis in Patients With Already Established Coronary Artery Disease or Events?

Because endothelial dysfunction plays an important role in the pathogenesis of atherothrombotic disease, it is not surprising that many studies have demonstrated a potential prognostic role of endothelial function in the coronary and peripheral circulation in secondary prevention. First evidence came from patients with nonobstructive coronary artery disease in whom significantly higher incidences of cardiovascular[120,121] and cerebrovascular events in those with impaired coronary vascular function were found.[122] Similarly, peripheral endothelial dysfunction assessed with FMD[123] and venous occlusion plethysmography predicted cardiovascular events in patients with coronary artery disease[124] and in patients after acute coronary syndromes.[125] In the setting of established coronary artery disease, patients with endothelial dysfunction have higher rates of adverse cardiovascular events compared with those with normal endothelial function,[126] and impaired FMD has been shown to be an independent predictor of in-stent stenosis after single-vessel coronary interventions.[127] In patients with advanced ischemic heart failure, endothelial function is a strong and independent predictor of 1-year mortality,[128] and in patients with graft vasculopathy (atherosclerosis associated with cardiac transplantation), normal endothelial function is associated with lower progression of coronary intimal thickening[129]; epicardial endothelial dysfunction independently predicts outcome in these patients.[130,131]

In acute myocardial infarction, microvascular endothelial dysfunction has especially been documented to be indicative of a poorer prognosis.[132–135] For example, no reflow on angiography strongly predicts 5-year mortality independently of infarct size in patients with acute ST-segment–elevation myocardial infarction.[136] Interestingly, no reflow might be reversible in some cases, which is associated with a better prognosis.[137]

Endothelial Function as a Contributor to Disease Progression?

Endothelial dysfunction in the periphery and in the coronary arteries is not only a marker for cardiovascular risk but also a contributor to the progression of atherosclerosis[100] and cardiovascular events. Interestingly, the atherosclerotic epicardial segments that show the most endothelial dysfunction are those with characteristics of vulnerable atherosclerotic plaques.[36] These segments are characterized by the loss of NO activity and increase in endothelin-1 activity,[138] the same segments more likely to progress to obstructive coronary artery disease.[139]

Importantly, microvascular dysfunction may contribute to the impaired regulation of myocardial perfusion by reducing the capacity to increase perfusion in response to exercise or mental stress, a circumstance that may lead to myocardial ischemia.[140] In the context of myocardial infarction, endothelial microvascular dysfunction is an important mediator of the event rather than just a consequence,[141] likely via reducing coronary blood flow by altering shear stress on the epicardial level, lowering endothelial function, and aggravating thrombus formation. Diabetes mellitus and the accumulation of risk factors in the metabolic syndrome, for example, have significant deleterious effects on myocardial perfusion and infarct size in patients with an acute infarction.[142–145] Moreover, patients with preprocedural impairment of microvascular function are more likely to have postprocedural microvascular impairment, procedure-related injury, and a worse outcome.[146] Thus, preexisting microvascular endothelial dysfunction leads to a greater vulnerability to myocardial injury, highlighting the potentially clinically relevant role of a dysfunctional microcirculation and damage.

Does Endothelial Function Identify Responders and Nonresponders to Therapy?

Many medical or lifestyle interventions can improve endothelial function and reduce cardiovascular events. For exam-

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ple, statin treatment significantly improves peripheral and coronary vascular function,[147] although not all studies were able to prove such an effect within a 6-month treatment period.[148,149] Of note, the impact on risk reduction despite this successful intervention is limited and is in the range of ≈20% to 45% in clinical trials. Even with the combination of all therapies proven to lower risk in secondary prevention (or primary prevention), some patients may develop later events and therefore are obviously not completely protected by their therapy.

Therefore, it is the ultimate goal to identify those patients who will develop future events despite therapy (to potentially escalate and intensify current treatment). One concept could be to measure the individual impact of therapy on endothelial function as a parameter of cardiovascular disease, targeting those with no improvement in vascular function. Important studies in this respect have recently been performed.

In a study in 251 Japanese men with newly diagnosed stable coronary artery disease and concurrent endothelial dysfunction (low FMD), FMD was repeated after 6 month of optimized individualized therapy. Those patients with persistently impaired FMD had significantly higher event rates in the follow-up period (26% over 31 months) compared with those with normal FMD (10%).[150] In a similar study, endothelial function was assessed in 400 postmenopausal hypertensive women without evidence of coronary artery disease at baseline and 6 months after blood pressure was treated to normotensive values. In those women whose endothelial function (FMD) had not improved (37.5%), there was a nearly 7-fold increase in cardiovascular events over the average follow-up of 67 month.[151]

Both studies convincingly demonstrate that patients who do not respond to the interventions with an improvement of endothelial function are at considerable risk of further events. More studies are needed to provide definitive answers to one of the most intriguing aspects of using endothelial function measurements.[152]

Nevertheless, one has to acknowledge that the pathophysiology of vascular dysfunction is extremely complex, probably differing among different vascular beds and between microvascular and macrovascular vessels. Thus, in the clinical endothelial function evaluation of the future, a more integrative approach should be considered, with the inclusion of peripheral macrovascular endothelial function and microvascular function measurements. An ideal test to identify a vulnerable patient should reflect and follow the disease state and thus should be abnormal with disease and possibly reversible with interventions (Table 4).

Table 4. Criteria for an Optimal Endothelial Function Test

Reflects disease state
Reversible with interventions
Mirrors coronary endothelial function
Improves risk stratification
Reproducible
Operator independent
Noninvasive (no or low risk for the patient)
Ease of use
Inexpensive

Is Improvement in Endothelial Function an Indicator of Successful Treatment?

It is probably a good sign when endothelial dysfunction is (partly) reversed with treatments. The first proof of this principle came from 2 controlled studies in 1995 in which cholesterol-lowering therapy (statins)[153,154] improved endothelial function. Statins now have convincing evidence for their beneficial effect on coronary and peripheral endothelial function,[147] likely because of their antiinflammatory and antioxidant properties and because of the restoration of the vascular NO bioavailability.[155] Since the first evidence in humans with statins, numerous interventions in a broad range of patients have demonstrated a beneficial effect on endothelial function. Most pharmacological intervention studies with an effect on cardiovascular risk factors also show improved endothelial function. For example, antihypertensive therapy in general[156,157] such as angiotensin-converting enzyme inhibitors,[158,159] angiotensin receptor blockers,[160] calcium channel blockers,[161] and certain β-blockers, particular the NO group containing the molecule nebivolol,[162] might reverse endothelial dysfunction; however, angiotensin-converting enzyme inhibitors seem to be particularly important.[157,163,164] Calcium channel blockers reduce calcium entry though L-type voltage-dependent channels of the vascular muscle cells, thus dilating coronary and other arteries. Additionally, some calcium channel blockers activate endothelial NO synthase or have antioxidative properties, thus increasing NO bioavailability.[165] The Evaluation of Nifedipine and Cerivastatin on Recovery of Coronary Endothelial Function-1 and -2 (ENCORE-1 and -2) trials showed that long-acting nifedipine consistently improved coronary endothelial function in patients with stable coronary artery disease and that the improvement persisted even after cessation of the drug.[148,166] Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are also preferred medications in diabetes mellitus.[167] Diabetes-modulating drugs like metformin[168] or glitazones[169,170] may also improve vascular function in patients with type 2 diabetes mellitus; however, the latter may have negative effects on cardiovascular risk,[171] thus limiting its use.

Not only pharmacological agents but also lifestyle factors and medications that increase the release of or prevent the degradation of endothelium-derived relaxing factors, NO in particular, and those that decrease the production of endothelium-derived constricting factors such as endothelin, among others, can improve endothelial function. Many interventions such as physical exercise,[172–174] weight reduction[175–177] (including bariatric surgery),[178,179] and enhanced external counterpulsation[180,181] and dietary interventions with foods rich in polyphenols, especially fruits, tea, and cocoa, have been demonstrated to be beneficial for microvascular or macrovascular endothelial function by increasing NO bioavailability.[182–185] An important lifestyle modification with an impact on endothelial function is smoking cessation. Smoking cessation clearly demonstrates a favorable effect on epicardial coronary endothelial function[80] that was not observed in the

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microvasculature.[185] This finding demonstrates the differences in macrovascular function and microvasculature function and their complex, incompletely understood interactions.

Although therapies with a proven benefit on morbidity and mortality in cardiovascular patients concordantly improve endothelial function, it is not certain whether the opposite is always true. For example, vitamin C or E and folic acid supplementation, praised for their antioxidative capacity and associated with significant acute improvement in endothelial function,[186] failed to show any benefit in the long term[187] or in cardiovascular disease prevention so far. On the other end, the pathophysiological information derived from studies on endothelial function is not correctly applied in clinical trials. Available evidence demonstrates that vitamin C can improve endothelial function[186] but also that this effect is obtained with concentrations much higher than those reached after oral administration.[188] Many studies evaluating pathophysiological aspects were performed in the setting of acute intravenous or intraatrial interventions. Moreover, the response to vitamins may be dependent on the presence of increased endogenous oxidative stress.[189] This kind of information should be evaluated carefully during the design of intervention studies. The abundant current trials with any kind of positive effects on the endothelium are pathophysiologically interesting, but care should be taken to extrapolate the findings to cardiovascular morbidity and mortality. Finally, it is of concern that the drug effects on endothelial function may be different according to which vascular bed is considered. Additionally, many lifestyle interventions, foods, and drugs have been shown to improve endothelial dysfunction in a population as a whole, but not necessarily in the individual patient, a fact which should be addressed in further studies.

Are Microvascular Function and Conduit Vessel Endothelial Function Comparable?

Although cardiovascular risk factors are associated with endothelial dysfunction in virtually every arterial bed, because of the different physiological role of conduit and resistance arteries, important differences should be considered. Whereas reduced NO release in response to stimuli plays a central role in the pathophysiology of endothelial dysfunction in the conduit arteries, NO in the microcirculation may primarily modulate tissue metabolism.[190] Furthermore, metabolic and other factors are becoming increasingly important in the regulation of microvascular function. Therefore, pharmacological tests inducing NO release might not reflect the physiological adaption of endothelial function in the microvasculature in response to exercise or ischemia.

The aforementioned differential effect of smoking on microvasculature and epicardial vasculature as outlined above might be only 1 example.[185] Furthermore, FMD is particularly sensitive to being impaired by traditional risk factors (eg, age, hypertension), whereas the peripheral arterial tonometry reactive hyperemia index (microvasculature) is more sensitive to metabolic risk factors, especially body mass index and diabetes mellitus[72,73] (and interestingly shows a paradoxical association with age in the Framingham cohort).[72] Microvascular and macrovascular dysfunction could also reflect different stages of vascular disease in that conduit artery endothelial dysfunction may be more important in patients with existing atherosclerosis and microvascular dysfunction may be an earlier indicator of risk. The fact that microvascular endothelial function and macrovascular endothelial function only show a weak (if any) correlation with each other[73] should caution against the extrapolation of findings in 1 circulation level to the other.

Given that macrovascular and microvascular endothelium is susceptible to different risk factors, both should be evaluated whenever possible.

Is There a Role of Endothelial Function in Drug-Development Programs?

For new drugs, the requirement by drug regulation authorities is to prove the principle by *primum non nocere* (first, do no harm); however, testing the effect of a certain drug on morbidity and mortality requires large sample sizes. Sometimes, such as for drugs with relatively small effects on the cardiovascular system or in children, such outcome trials are not feasible at all. Clinical endothelial function evaluation is of potential value in reassessing the risk of drug-development programs, especially as it becomes more challenging to choose novel agents for clinical use.[191] With endothelial function as a mechanistic surrogate integrating various types of cardiovascular risks,[49,191] the sample size can be significantly smaller compared with clinical end-point trials.[52] Furthermore, endothelial function may respond rapidly to therapies (within hours, days, or weeks), long before the effects on clinical outcomes are seen. Thus, the impact on endothelial function may give important signals of efficacy or, more important, may warn of potential harm. Therefore, endothelial function not only is a valuable measure to assess drug efficacy on surrogate end points but also may play an important potential role in the evaluation of drug safety, as exemplified in the recently completed Dalcetrapib's effects on vascular function study (dal-VESSEL) study,[192] notably the first multicenter study to use FMD as outcome measure. For certain studies, a multimodality approach, which includes peripheral endothelial function measurements, may be of particular value.[193]

What Kind of Studies Do We Need in the Future?

As outlined above, endothelial function measurement may differentiate responders from nonresponders to therapy.[151] In secondary prevention, studies demonstrate that patients who do not respond to interventions with improved endothelial function are at a considerable risk for further events. These early data suggest that therapy guided by individual endothelial function measurements might be feasible in these settings, but larger studies in this respect are needed to answer the question of whether endothelial function–guided therapies help to improve outcomes.

In primary prevention, it is still unknown whether endothelial function should be assessed in apparently healthy individuals at low risk from traditional risk factors. To address this issue, endothelial dysfunction, which depicts mechanisms at the core of atherosclerosis and its complications, could be chosen for future similar studies, with different medical and lifestyle interventions to be tested. Designing

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such a trial would require very careful consideration of which noninvasive test or combination of tests of endothelial function should be included. If such results prove positive, there would be a good rationale to implement endothelial function testing in everyday clinical practice.

Currently, clinical guidelines and risk management for prevention are based on the risk factors established in the Framingham study and certain cardiovascular surrogates such as carotid IMT and coronary calcium.[118,119] However, the Framingham score and other scores provide inconsistent results when applied to different populations,[194] and adjustment based on different populations might be needed. Additionally, the Framingham score is limited to the fact that risk factors were collected years ago, when, for example, no statin therapy was available and most people smoked or were exposed to secondhand smoke. The effect of a changing environment might be better depicted by endothelial function assessments. With the assumption that endothelial function provides an integrated functional risk assessment, the question of whether endothelial function might be a better predictor for cardiovascular events than the actual scoring systems is intriguing and should be tested with larger-scale studies.

When using the Framingham Risk Score, we are aware how to deal with patients in the high- or low-risk category. However, many patients end up having intermediate risk; for these patients, the recommendation are less clear. As demonstrated by the studies discussed above, reclassification of patients with intermediate risk according to their endothelial function seems to be feasible and reasonable, although further studies in this area are required.

Conclusions

In the past decade, many studies have suggested that the noninvasive assessment of endothelial function may provide important information for individual patient risk, progress, and guidance of therapy (in addition to its well-established role in clinical research). This is underscored by the low risk of the tests and the valuable information that can be derived from them. Thus, further research should be directed at determining whether measurement of endothelial function can be used to guide treatment, change outcomes, and to ascertain whether detection of endothelial function will be useful in clinical arena.

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