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REVIEW

The endothelial cell in health and disease: its function, dysfunction, measurement and therapy

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Endothelial cells have numerous endocrine functions and contribute to a variety of processes, including penile erection and vasodilation. Endothelial dysfunction is associated with cardiovascular risk factors and has been implicated in the pathogenesis of atherosclerosis and ED. This study reviews endothelial function, in addition to endothelial dysfunction and its role in atherosclerosis and ED. Measurement of endothelial function is reviewed, including catheter-based methods, venous occlusion plethysmography, high-frequency ultrasound, peripheral arterial tonometry, digital pulse amplitude tonometry, digital thermal monitoring, the L-arginine test and measurement of compounds released by endothelial cells. Therapy and medications that improve endothelial function are reviewed. As the scientific community learns more about the importance of the endothelium, it is increasingly important for the clinician to understand endothelial function, dysfunction, measurement of endothelial function and therapies that affect this remarkable cell type.
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Introduction

The endothelium has many endocrine functions and contributes to a variety of processes, including penile erection. Endothelial dysfunction occurs early in atherosclerosis,[1–5] and has been implicated in the pathogenesis of ED.[4,6–11] Cardiac risk factors are prevalent in patients with ED[12] and correlate with endothelial dysfunction, including hypertension,[13] hypercholesterolemia,[3,14,15] diabetes,[6,15] smoking[3,16–18] and age,[18] as well as with atherosclerosis.[3,19–25] This study reviews normal endothelial function, as well as endothelial dysfunction in general and related specifically to ED. A variety of methods to measure endothelial function and therapies that improve endothelial function are reviewed. As endothelial dysfunction becomes more important to clinical practice, the clinician should understand its pathophysiology, its measurement and therapies that affect the endothelial cell.

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Endothelial cell function (physiology)

Endothelial cells occupy a pivotal location between blood and tissue, which facilitates their involvement in numerous physiological processes. Blood vessels consist of a layer of smooth muscle surrounding an inner layer of endothelium (Figure 1). In addition to providing a selectively permeable barrier to blood, endothelial cells are vital to maintaining a physiological equilibrium relating to the processes of inflammation, platelet aggregation, thrombosis and vascular smooth muscle proliferation.[2] Endothelial cells also modulate vascular tone and blood flow and, in doing so, have profound effects on the overall function of the cardiovascular system.[2]

The endothelium responds to various humoral, neural and mechanical stimuli by releasing both contractile and relaxing signals that affect the underlying smooth muscle and thus, vascular tone.

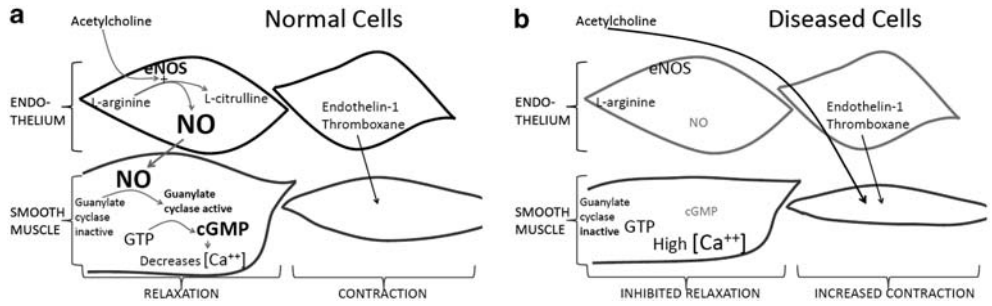

Figure 1 Schematic of endothelial function and dysfunction. (**a**) With normal endothelial cells, acetylcholine initiates vasodilation by stimulating eNOS to catalyze the conversion of L-arginine to L-citrulline and NO. NO diffuses to adjacent smooth muscle cells and activates guanylate cyclase, which converts GTP to cGMP. cGMP decreases intracellular calcium concentration and muscle tone resulting in vasodilation. Conversely, endothelin-1 and thromboxane stimulate smooth muscle contraction and vasoconstriction. (**b**) In endothelial dysfunction, production and/or diffusion of NO are inhibited. Without NO in the smooth muscle cell, guanylate cyclase remains inactive, cGMP levels remain low and calcium concentration remains high. When the NO-cGMP pathway is inhibited, the balance between vasodilation and vasoconstriction shifts to vasoconstriction. In severe endothelial dysfunction, acetylcholine acts directly on smooth muscle, stimulating vasoconstriction. eNOS, endothelial nitric oxide synthase; GTP, guanosine triphosphate; cGMP, cyclic guanosine monophosphate; [Ca^{++}], calcium ion concentration.

Endothelial cells are influenced by shear force, by platelets and the coagulation system, and by hormones and neurotransmitters.[2,26] The endothelium, in turn, influences vascular tone through a variety of signals, including the vasoconstrictors angiotensin II, endothelin (ET)-1 and thromboxane, as well as vasodilators prostacyclin and NO.[2,26]

NO-dependent vasodilation

Endothelial cells contribute to the regulation of blood flow, in part through NO-dependent vasodilation (Figure 1).[2,26,27] NO-dependent vasodilation is initiated when agonists (such as acetylcholine and shear stress) activate the endothelial cell's phosphoinositol pathway and increase cytosolic calcium levels. In the endothelial cell calcium binds to calmodulin, which then activates endothelial nitric oxide synthase (eNOS) to form NO from its precursor substrate, L-arginine. NO diffuses to the adjacent smooth muscle cells where it activates soluble guanylate cyclase to increase cyclic guanosine monophosphate (cGMP) levels. cGMP relaxes vascular smooth muscle by decreasing calcium levels through inhibition of the phosphoinositol pathway. In summary, agonists stimulate eNOS to produce NO, which increases smooth muscle cGMP and reduces smooth muscle calcium levels and tone. NO continuously fluctuates at low concentrations and therefore constantly influences the vascular tone.[2]

NO-dependent penile erection

The endothelium has a larger role in penile erection than simply influencing blood flow from within the internal pudendal and helicine arteries that supply erectile tissue. The corpus cavernosum is made of interconnected smooth muscle that is lined by vascular endothelial cells. Similar to the systemic vasculature, NO from endothelial cells diffuses into erectile smooth muscle, where it increases cGMP levels, decreases calcium concentration and reduces muscle tone.[11,27]

Endothelial function has a more important role in penile erection than in other vascular beds. To sustain an erection, the cavernous and helicine arteries dilate up to 80%, whereas most conduit arteries dilate only about 15% in response to flow-mediated dilation (FMD).[4] Moreover, engorgement of the trabecular spaces with blood compresses the venous system and prevents venous outflow.[4] In contrast, endothelial cells have little influence on most other venous networks. By inducing substantial arterial dilation, relaxing trabecular smooth muscle and compressing the venous circulation, endothelial cells are vital to the erection process.[11]

Endothelial cell dysfunction (pathophysiology)

Although the term 'endothelial cell dysfunction' can refer to disruption of any of the processes that require healthy endothelial cells, it is most commonly used to describe abnormal endothelial-dependent smooth muscle relaxation (that is, vasodilation) due to impairment of the NO-cGMP pathway.[27]

Impaired NO-dependent vasodilation

In 1980, Furchgott and Zawadzki discovered that a vessel with intact endothelial cells dilated when exposed to acetylcholine; however, if the vessel was denuded of endothelial cells, acetylcholine would paradoxically induce vasoconstriction.[2,5,26] Acetylcholine is an endothelial-dependent vasodilator

through the NO-cGMP mechanism, but in the absence of endothelial cells (or absence of normal endothelial cells), acetylcholine has a constrictor effect on smooth muscle (Figure 1). When acetylcholine was infused intravenously, normal controls showed a vasodilatory response, but responses were reduced or vasoconstrictive in patients with hypertension,[13] diabetes,[15] hypercholesterolemia,[14] congestive heart failure[28] or atherosclerosis.[5,21,23–25] Similarly, increases in flow subject the endothelium to increased shear stress, which stimulates the NO-cGMP pathway, so that FMD is also dependent on endothelial cells. Abnormal FMD has been shown in patients with cardiac risk factors and in atherosclerosis.[3,5,16–20,22,29] Importantly, many of these studies also showed that vasodilation to nitrates, which act directly on smooth muscle in an endothelial-independent manner, was preserved.[13–16,18,21,23,24] Moreover, N^G-monomethyl L-arginine (L-NMMA), a NO synthase inhibitor, blunted the vasodilatory response to acetylcholine and FMD, but did not affect nitroprusside-induced vasodilation.[30,31]

Endothelial dysfunction contributes to atherosclerosis

The NO-cGMP pathway becomes impaired before the development of atherosclerosis and its impairment likely contributes to the pathogenesis of atherosclerosis. In the absence of NO, platelets are better able to adhere, aggregate and release platelet-derived growth factor; leukocytes are better able to adhere, promote a chronic inflammatory response and stimulate the growth of underlying cells; more superoxide is available to oxidize LDL; and the vessel is more prone to constriction or 'spasm'.[2,5] Furthermore, endothelial dysfunction likely contributes to clinical syndromes of myocardial ischemia, including stable angina (by paradoxical vasoconstriction that increases stenosis resistance and reduces coronary flow reserve), unstable angina (by the lack of inhibition to platelet deposition and aggregation and paradoxical vasoconstriction) and especially variant angina (by paradoxical vasoconstriction resulting in coronary spasm).[5] Moreover, endothelial dysfunction of the microvasculature has been implicated in cardiac syndrome X, a condition characterized by typical angina, ST-segment depression during exercise electrocardiogram stress testing and normal coronary arteries by angiography.[32] Patients with cardiac syndrome X have evidence of endothelial dysfunction[33] and showed subendocardial ischemia and chest pain in response to adenosine,[34] which vasodilates the microvasculature in a partially endothelial-dependent manner.[35] The critical role of endothelial cells in relation to atherosclerosis and cardiovascular events is becoming apparent.

Endothelial cell dysfunction and erectile dysfunction

The importance of endothelial cells and the NO-cGMP pathway extends to penile erection. Compared with healthy controls, patients with ED and without cardiac risk factors had impaired FMD in the brachial artery, abnormal penile Doppler studies and showed improvement in ED after $\geqslant 2$ months of sildenafil.[4] As sildenafil (and other PDE5 inhibitors) works by inhibiting the breakdown of cGMP, the authors concluded that these patients without cardiac risk factors had a vascular origin of ED.[4] Compared with patients without ED, patients with ED had a blunted blood pressure and platelet aggregation response to L-arginine (see below), indicative of an impaired NO-cGMP system.[6] Corpus cavernosum tissue from patients with diabetes (*in vitro*) showed impaired relaxation in response to autonomic nerve simulation and to acetylcholine, but not to the endothelial-independent vasodilators nitroprusside and papaverine, suggesting endothelial dysfunction.[7] ET-1 (a marker of endothelial cell activation, see below) was measured in several groups of men with versus without ED, and in men with ED with versus without signs of and risk factors for cardiovascular disease.[8,9] Compared with men without ED, patients with ED had significantly higher plasma concentrations of ET-1.[8] Among men with ED, concentrations of ET-1 were not significantly different in peripheral and cavernous body blood.[8] ET-1 concentration was not different in men with ED with versus without signs and risk factors for cardiovascular disease.[9] Together, the authors concluded that independent of cardiac risk factors and overt vascular disease, men with ED have elevated markers of endothelial cell activation (ET-1); these markers are likely to be of systemic origin and indicate the initial stage of diffuse vascular disease; moreover, endothelial dysfunction may be involved in the pathogenesis of ED in patients with and without diabetes. Collectively, these studies indicate that impairment of the NO-cGMP system has a critical role in the etiology of ED and that patients with ED have systemic endothelial dysfunction.

Measuring endothelial function

Peripheral endothelial function correlates with coronary endothelial function,[36–38] reflecting the systemic nature of atherosclerosis. Moreover, in certain patient populations, peripheral endothelial dyfunction predicts the presence of coronary artery disease,[38,39] cerebrovascular events[40] and cardiovascular events.[29,39,41–54] Therefore, measuring peripheral endothelial function in clinical practice may provide important prognostic information regarding

the presence of coronary artery disease. A noninvasive test that aids in cardiovascular risk stratification would significantly impact patient care in cardiology and potentially in ED. An ideal test would be noninvasive, easy to administer, reproducible and standardized between laboratories. An ideal test would enhance the predictive capacity, especially in intermediate-risk patients, and would show improvement in response to therapies that reduce cardiovascular risk.[55,56] No test currently fits all these criteria, nor does any test have a universally agreed upon cutoff to define abnormal on the basis of large-scale trials; however, the field is moving toward a test that is simple enough to perform in the office (Figure 2). Most current tests measure NO-dependent vasodilation, but measuring platelet aggregation and circulating endothelial-derived molecules also reflects endothelial function.

Eliciting endothelial-dependent vasodilation

Endothelial-dependent vasodilation can be elicited with IV agents (that is, acetylcholine, bradykinin) or with reactive hyperemia (shear stress). Reactive hyperemia is frequently elicited by inflating a blood pressure cuff around the arm to a suprasystolic pressure for 5 min, which occludes the brachial artery. The distal tissue becomes ischemic, incurs oxygen debt and accumulates metabolic waste products. When the cuff is released, blood flow resumes. Oxygen debt in distal tissues and endothelium stimulates local vasodilation, which in turn increases blood flow through the more proximal brachial artery (reactive hyperemia). Increased blood flow, hyperemia, causes increased shear stress, which stimulates NO-dependent vasodilation in the brachial artery. Inducing reactive hyperemia with a 5-min cuff occlusion generally increases blood flow initially by 500–600% from baseline and, in healthy controls, increases brachial artery diameter by a maximum of about 7–15% from baseline after 1 min.[3,16,17,22] Endothelial-dependent vasodilation is often compared with nonendothelial-dependent vasodilation, most commonly with nitroglycerin, which acts directly on vascular smooth muscle.

Catheter based

There are two catheter-based methods that measure endothelial function: one, using acetylcholine, is currently limited to research studies and the other, coronary vasodilatory reserve, is commonly used. Coronary vasodilatory reserve (or coronary flow reserve) is the ratio of hyperemic to basal average peak flow velocity.[57] Hyperemia is typically achieved with intracoronary adenosine, which dilates the microvasculature and is partially endothelial dependent.[35] Coronary vasodilatory reserve is commonly used for patients with coronary artery disease to evaluate the physiological significance of intermediate severity stenoses, serial stenoses and other lesions of unclear significance. Coronary vasodilatory reserve also has a class IIb indication for the evaluation of patients with angina without an apparent angiographic lesion to investigate the possibility of cardiac syndrome X.[32,57]

In research studies, endothelial function is measured by infusing graded concentrations of acetyl-

METHOD	NON-INVASIVE	SIMPLE TO USE	REPRO-DUCIBLE	STANDARD-IZED	PREDICT CAD / CV EVENTS†
CATHETER-BASED	-	-		+	+++
HIGH-FREQUENCY ULTRASOUND	+	-	+++	-	+++
VENOUS OCCLUSION PLETHYSMOGRAPHY	+	-		-	+++
PULSE WAVEFORM ANALYSIS	+	+	+	-	
DIGITAL PULSE AMPLITUDE TONOMETRY	+	+++		+	+
DIGITAL THERMAL MONITORING	+	+++		+	+
L-ARGININE TEST	+	-		-	
MEASURING CIRCULATING ADHESION MOLECULES	+	+		+	+

Figure 2 Characteristics of various methods of measuring endothelial function. + + +, superior characteristic or >2 supportive trials with high-quality methodology; +, positive characteristic or supportive evidence in literature; blank, not extensively studied or unknown; −, does not have characteristic; CAD, coronary artery disease; CV, cardiovascular. †References for catheter-based,[40–44,49] ultrasound,[29,39,45,46,51,52] venous occlusion plethysmgraphy,[47,48,50,54] digital pulse amplitude tonometry,[68] digital thermal monitoring[71] and measuring circulating adhesion molecules.[75,76]

choline into a coronary artery and measuring changes in vessel diameter with quantitative angiography and measuring changes in blood flow with Doppler flow wires.[57] This method predicts cerebrovascular events[40] and long-term cardiovascular events,[41–44,49] and is considered the 'gold standard' for evaluating endothelial function. However, the invasive nature of catheter-based methods is not conducive for a screening test or for serial evaluations, hence this method is limited to patients undergoing cardiac catheterization for the evaluation of chest pain.

High-frequency ultrasound

Reactive hyperemia can be evaluated noninvasively using high-frequency ultrasound, which has proven accuracy and reproducibility.[3,16,22,58] It is widely used in clinical research trials and predicts coronary artery disease,[45] postoperative cardiac events[46] and long-term cardiovascular events.[29,39,51–53]

Attention to detail is essential to ensure accurate and reproducible measurements using high-frequency ultrasound; a summary of the technique is presented.[3,22,58] Testing should be performed by an experienced sonographer, in the morning, after an overnight fast, in a temperature-controlled room ($\sim 22\,^{\circ}C$), with the subject supine, and after a rest period so that a steady baseline is reached. The subject's arm should be extended with the hand supinated, then immobilized, exposing the brachial artery in a constant position for imaging. The brachial artery should be located 3–7 cm superior to the antecubital crease. The skin should be marked for a consistent measuring point and the artery scanned in longitudinal section. A 7 MHz linear array transducer should be used with settings to optimize the blood-vessel wall interface. Image depth should be 4 cm and the focus set to the depth of the near wall. Vessel diameter should be measured from the near to the far 'm' line (the media–adventitia interface) and blood flow measurements should be taken from the artery's center (Figure 3) at end-diastole (the onset of the QRS complex).

Numerous studies have used high-frequency ultrasound; however, this method currently lacks standardization between laboratories. Corretti *et al.* [22] evaluated the blood flow and vessel dilation response to various durations of occlusion over time to better characterize this technique. Cuff occlusions (endothelial dependent) of 1 and 3 min were insufficient to elicit statistically significant vasodilation in healthy controls, whereas a 5 min occlusion led to a peak diameter increase of $12.6 \pm 5.7\%$ at 1 min, and arterial diameter remained significantly dilated compared with baseline throughout 20 min. After nitroglycerin administration (endothelial independent), diameter was significantly dilated compared with baseline throughout 10 min, and a peak diameter increase of $19.4 \pm 5.9\%$ occurred at 4–5 min. A 5-min occlusion elicited similar increases in blood flow in both healthy controls and patients with coronary artery disease; however, brachial artery diameter increased significantly in controls, but not in patients with coronary artery disease. In conclusion, a 5-min occlusion should be used, flow should be measured immediately after cuff release and diameter should be measured 1 min after cuff release.

High-frequency ultrasound is noninvasive, enables testing of young subjects at an early preclinical stage and detects reversibility. However, it is operator dependent, technically challenging, requires specific training and equipment and currently lacks standardization between laboratories.

Venous occlusion plethysmography

Venous occlusion plethysmography (including strain-gauge plethysmography) is based on the principle that during occlusion of venous return, the rate of swelling of the forearm can be used to assess the rate of arterial inflow. A cuff is inflated to 40 mm Hg, which occludes venous return but does not affect arterial inflow. The

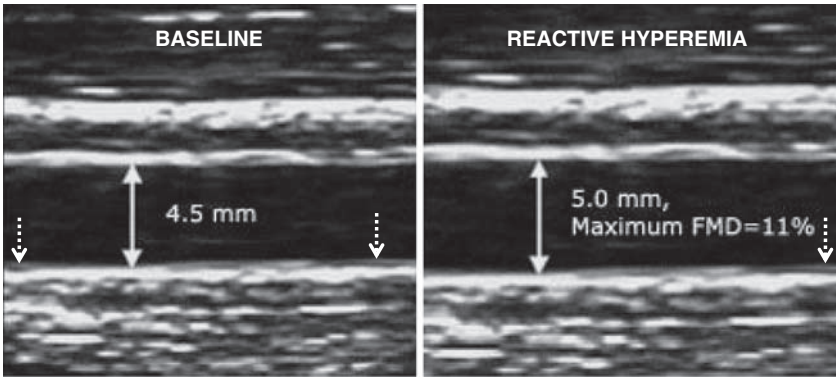

Figure 3 Ultrasound image of brachial artery before and after reactive hyperemia. Right: normal reactive hyperemia induced flow-mediated dilation (FMD) of 11% from baseline (left). Solid arrows indicate media–adventitia interface ('m'-line). Dotted arrows indicate the blood-endothelial cell interface. Reprinted with permission from The Cardiovascular Risk in Young Finns Study.[99]

rate of swelling distally can be measured by water displacement or can be calculated from changes in forearm circumference measured by a strain gauge. Either reactive hyperemia or intra-arterial infusion of vasoactive agents can be used. This method measures blood flow, not arterial diameter, depends on conditions at baseline and lacks standardization between laboratories.[59] Despite these limitations, venous occlusion plethysmography predicted cardiovascular events.[47,48,50,54]

Pulse waveform analysis/peripheral arterial applanation tonometry

Applanation tonometry was originally designed to measure intraocular pressure, but has been adapted to measure arterial pulse (pressure) waveforms. The tonometer, a device resembling a short pencil, is positioned over the radial artery maximal pulsation to minimally flatten the arterial wall. Intra-arterial pressure influences the electrical resistance of a small piezoelectric crystal within the tip of the tonometer, allowing the recording of the pressure waveform.[60] Differently sized vessels affect pulse waveforms and blood pressure differently.[61] Large vessels (aorta) influence pulse wave velocity and pulse pressure (the difference between systolic and diastolic pressure) because of their elastin and collagen content, which are altered by aging and disease. Medium-sized vessels (brachial) also affect pulse wave velocity but smooth muscle influences medium-sized vessel compliance. Dependent on smooth muscle tone, distal branch points reflect the pulse wave and affect its contour. Smooth muscle tone in resistance arterioles influences the mean arterial pressure. With simultaneous blood pressure recording and pulse wave analysis, a computer formulates the compliance and caliber of the large and/or small vessels and synthesizes data into an 'augmentation index'. Different devices may report an augmentation index for proximal vessels, distal vessels or both. Vessel compliance and tone are influenced by NO and so reflect endothelial function. Pulse waveform analysis often utilizes inhaled albuterol, a beta$_2$-agonist, to stimulate the release of NO from endothelial cells and elicit a change in vessel compliance.[62] Pulse waveform analysis was associated with forearm vascular resistance as assessed by ultrasonography,[63] and was more significantly correlated with cardiovascular risk factor score than ultrasound measured FMD.[64] Salbutamol (similar to albuterol) was associated with a significantly smaller decrease in augmentation index in 12 patients with angiographically proven coronary artery disease compared with 10 healthy, age-matched controls.[60] Peripheral arterial applanation tonometry is noninvasive and simple to use, but more testing is needed before its widespread use as a clinical screening tool can be recommended.

Digital pulse wave amplitude/digital pulse amplitude tonometry

A variation on pulse waveform analysis, the Endo-PAT2000 (Itamar Medical Limited, Caesarea, Israel), measures pulsatile volume changes within the distal phalanx to assess endothelial function. A specially designed finger plethysmograph fits around the distal phalanx and imparts a uniform pressure (70 mm Hg) to prevent distal venous distention and unload arterial wall tension (Figure 4). With normal endothelial function, reactive hyperemia causes an increase in digital pulsatile volume changes, which the sensor measures and the instrument filters, displays and stores.

Digital pulse amplitude tonometry measurement of the reactive hyperemia response correlated with high-frequency ultrasound,[65] with the presence of multiple cardiovascular risk factors[65,66] and with coronary endothelial dysfunction.[67] Rubinshtein *et al*.[68] assessed endothelial function in 270 patients with the Endo-PAT2000 and recorded MACE (cardiac death, myocardial infarction, revascularization and cardiac hospitalization) over a median of 5.8 years. Endo-PAT2000 measurements were associated with MACE and independently predicted cardiac hospitalizations beyond the Framingham risk score. Digital pulse amplitude tonometry is standardized, noninvasive, easy to use and independently predicts cardiac hospitalizations. However, as Celermajer points out,[55] its relationship with most cardiovascular risk factors was relatively weak and it has a paradoxically positive relation with age. Large-scale studies are underway to determine its utility in relation to the existing tests for cardiac risk stratification.

Digital thermal monitoring

A somewhat similar device, Endothelix's VENDYS, is also placed on the fingertip and assesses

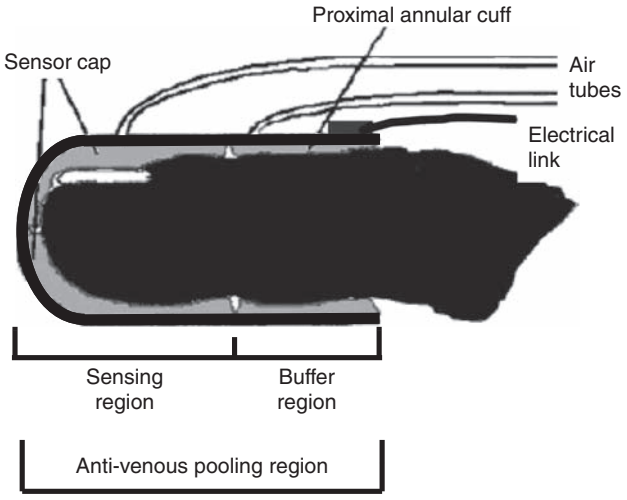

Figure 4 Peripheral arterial tonometry finger probe. Cross-sectional image of digital pulse amplitude tonometry probe (image courtesy of Itamar Medical Ltd.).

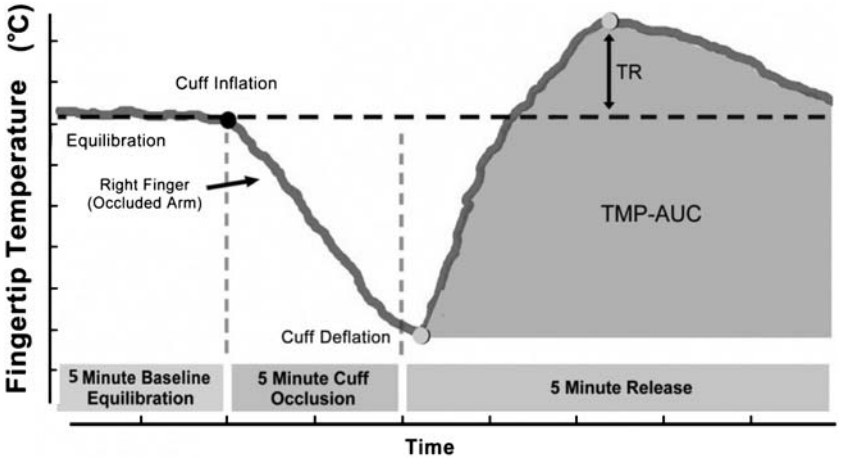

Figure 5 Digital thermal monitoring of vascular reactivity. Digital temperature over time measured by digital thermal monitoring. After cuff deflation, normal endothelial function manifests as a temperature rebound above the previous baseline. TR, temperature rebound; TMP-AUC, temperature area under the curve (image courtesy of Endothelix, Inc.).

endothelial function through digital thermal monitoring. After a baseline temperature is established, a cuff is used to occlude blood flow in a standard manner and the digital temperature decreases. After 5 min, when the cuff is released and reactive hyperemia ensues, a normal response is manifest by a 'temperature rebound' above the previous baseline, whereas the temperature rebound is blunted or absent in patients with endothelial dysfunction and inhibited vasodilation (a normal response is shown in Figure 5). Patients with coronary heart disease and patients with an increased Framingham risk score had a lower temperature rebound compared with normal controls.[69] Digital thermal monitoring was associated with coronary artery calcium, with obstructive coronary artery disease assessed by computed tomography[70,71] and with abnormal myocardial perfusion imaging measured by summed stress score.[72] Moreover, digital thermal monitoring reflected a response to therapy.[73] The VENDYS device is noninvasive, easy to use and standardized. Large-scale studies are ongoing to better delineate its role in cardiac risk stratification.

L-arginine test induces changes in blood pressure and platelet aggregation

When L-arginine is administered intravenously, it undergoes endothelial-dependent metabolic conversion to NO. The increase in NO levels changes blood pressure and platelet aggregation, the degree of which correlates with endothelial function. Giugliano *et al.*[74] investigated the responses over time to different doses of L-arginine in healthy controls and in patients with diabetes, hypercholesterolemia or hypertension. Three grams of L-arginine produced the maximal hemodynamic effect, which occurred 10 min after the intravenous bolus. Blood pressure and antiplatelet responses declined progressively with age, such that blood pressure decreased 8 ± 1 mm Hg in controls <30 years old and only 2.8 ± 0.4 mm Hg in controls >60 years old. Compared with age-matched controls, the blood pressure response was significantly blunted in patients with diabetes or hypercholesterolemia, but not in those with hypertension. L-arginine decreased platelet aggregation in controls; in contrast, the response to L-arginine was blunted in patients with hypertension and was paradoxically increased in patients with diabetes or hypercholesterolemia. The L-arginine test requires hemodynamic monitoring, intravenous access and a blood draw, and varies significantly with age, but may prove useful with further testing.

Measurement of compounds released by endothelial cells

Activation of endothelial cells can be assessed by measuring compounds released by endothelial cells, including inflammatory cytokines and adhesion molecules vascular cell adhesion molecule, intercellular adhesion molecule (ICAM), P-selectin and ET-1. Levels can be determined by commercially available enzyme-linked immunosorbent assay. These markers may be elevated in subjects with atherosclerosis, dyslipidemia, hypertension, diabetes and obesity.[9,56,75,76] A nested case–control analysis[75] reported that ICAM predicted future myocardial infarction in healthy men, and a similar case–control analysis[76] reported that ICAM predicted coronary artery disease. The simplicity and safety of this method hold promise for clinical use.

Summary of the methods for measuring endothelial function

A variety of methods measure endothelial function with the potential to be used as a screening test for cardiac risk stratification. Catheter-based measure-

ment with acetylcholine remains the 'gold standard', but is not conducive to large-scale clinical screening because it is invasive, relatively high risk and requires skilled technicians. High-frequency ultrasound is noninvasive and has proven accuracy and reproducibility; however, the need for skilled personnel and longer testing time may preclude its use as a clinical screening modality. Venous occlusion plethysmography, peripheral arterial applanation tonometry, the L-arginine test and measuring compounds in the serum, each has advantages and disadvantages. Digital pulse amplitude tonometry and digital thermal monitoring are each standardized, simple to use and feature a noninvasive fingertip sensor. Although additional trials are underway, each method has already shown an association with more established measures of endothelial function, with cardiovascular risk factors and indicators of atherosclerosis. Any method would be a valuable screening test if validated by large-scale prospective studies and abnormal cutoffs or ranges are better defined. Perhaps a combination of methods will be the most effective for cardiac risk stratification.

At this time, there are no accepted guidelines for when and under what circumstances to conduct endothelial function testing; however, internists, cardiologists and urologists have begun using the Endo-PAT2000 and VENDYS devices in clinical practice. With a few exceptions,[63,66] nearly all published data describe testing that was carried out on relatively small numbers of high-risk patients and relatively small numbers of controls in the morning, in a fasting state, after the avoidance of vasoactive medications, caffeine and alcohol. Until large-scale, prospective testing has been carried out on other patient groups and under various conditions, test results should be interpreted with caution and repeat testing under optimal conditions may reduce false-positive results. Prospective, randomized trials are needed to determine the applicability of endothelial function testing to various groups of patients and to better define abnormal cutoff values or ranges. In the interim, testing endothelial function is reasonable for patients with intermediate or high-risk clinical markers in whom the presence of atherosclerosis is unknown, including patients with ED, clinical claudication, diabetes or other cardiac risk factors. Patients with chest pain should undergo traditional evaluation and testing according to current algorithms and accepted guidelines with a better-defined relation to coronary artery disease (stress tests or coronary angiography).

Measuring endothelial function in relation to ED

In addition to serving as a surrogate marker for systemic atherosclerosis, endothelial function testing is being used in ED research trials to diagnose vasculogenic ED. Penile duplex ultrasonography is commonly used to measure vascular parameters after pharmacological stimulation. In this setting, cavernous artery peak systolic velocity, end-diastolic velocity and acceleration time correlate fairly well with patients' vascular status.[77–79] The commonly used pharmacological stimulants, papaverine, prostaglandin E1 and phentolamine, act directly on smooth muscle independently of the endothelium. Although PDE5 inhibitors are not the preferred agents because they are more time-consuming and fewer patients achieve an erection, as PDE5 inhibitors act by decreasing the breakdown of NO, their use targets the endothelial-mediated pathway. Although numerous studies have evaluated the cavernous arterial response to PDE5 inhibitor therapy, only a few small studies[80,81] have examined the use of PDE5 inhibitors for the diagnosis of vasculogenic ED. Perhaps PDE5 inhibitors can enhance the diagnostic capacity.[80] Ucar *et al.*[82] reported that the combined use of brachial artery FMD and carotid artery intima-media thickness diagnoses vasculogenic ED with high accuracy. Furthermore, among patients with vasculogenic ED, brachial artery FMD differentiated between arterial insufficiency and cavernous veno-occlusive disease.[82] Endothelial-dependent vasodilation of the cavernous arteries is being elicited using a special sphygmomanometric cuff inflated at the base of the penis to suprasystolic pressure for 5 min, similar to blood pressure cuff-induced FMD of the brachial artery. Using these methods Mazo *et al.*[83,84] showed that a PDE5 inhibitor increased FMD in both penile and brachial arteries. Interestingly, patients with arteriogenic ED (assessed by Doppler ultrasonography with intracavernosal prostaglandin E1 injection) showed greater increases in cavernous versus brachial artery dilation in response to a PDE5 inhibitor and no correlation was found between cavernous and brachial arteries.[83,84] This report[84] indicates that for diagnosing arteriogenic ED, measuring FMD in cavernous arteries is highly sensitive and specific and is superior to measuring FMD in the brachial artery. Various methods of endothelial function measurement are being used in research studies to establish and further elucidate the link between endothelial dysfunction and ED[4,6,8,9,85–88] with the goal of establishing a simple diagnostic test for vasculogenic ED.[80–84,89–91]

Clinical implications of endothelial function testing

The close correlation of peripheral endothelial function with coronary artery disease is consistent with our current understanding of the pathogenesis of atherosclerosis, whereby endothelial dysfunction occurs early and contributes to disease progression

systemically. Endothelial dysfunction, then, is not an isolated insult but rather a manifestation of atherosclerosis that should alert the physician to the likelihood of coronary artery disease and future cardiovascular events. Similarly, vasculogenic ED is usually a sign of endothelial dysfunction and a marker of systemic atherosclerosis.

Patients found to have endothelial dysfunction are no longer only 'at risk' for developing atherosclerosis, but rather the disease process has already

THERAPY	AFFECT ON ENDOTHELIAL FUNCTION	IMPROVE CV ENDPOINT	IMPROVE ED
Smoking cessation	FMD was higher in former smokers vs. current smokers, especially in men (p=0.001). (16)	Yes (100)	unknown
Low cholesterol diet	A high-fat meal (50 gm of fat) decreased FMD from a baseline of 20% to 12%, 10%, and 8% at 2, 3, and 4, hours, respectively (p<0.001). A low-fat meal did not significantly decrease FMD (observer-blinded, randomized). (101)	Yes (102)	maybe (103)
L-arginine supplementation	L-arginine increased FMD in patients with impaired FMD at baseline (<7%) but had no effect on patients with baseline FMD >7% (meta-analysis of randomized, placebo-controlled trials). (104)	unclear (105)	unknown
Antioxidants	Ascorbic acid (2 gm orally) significantly improved FMD (2.0% to 9.7%) in patients with coronary artery disease vs. placebo (p=0.003). (106)	No (94)	unknown
	Vitamins C and E blunted the decrease in FMD following a high-fat meal (observer-blinded, randomized). (101)		
Aged garlic extract	Aged garlic extract (250mg) plus Vitamin B12, folic acid, Vitamin B6, and L-arginine (100mg) daily for 1 year increased digital temperature rebound vs. placebo (p=0.001) (randomized, double-blind, placebo-controlled) (73)	maybe (73)	unknown
Estrogen	Estradiol (1 or 2 mg / day) increased brachial artery FMD vs. placebo (p<0.05) (double-blind, placebo-controlled, cross-over). (107)	No (95)	unknown
PDE5 inhibitors	Main mode of action. Commonly used for ED, chronic daily dosing improves peripheral endothelial function, FDA approved for pulmonary hypertension, appears to benefit patients with CHF (especially with secondary pulmonary hypertension). (92,93)	maybe (92,93)	Yes (92,93)
Statins	5.5 months of lovastatin improved coronary response to acetylcholine vs. lipid-lowering diet alone (p=0.004) (randomized, double-blind, placebo-controlled). (108)	Yes (110)	unclear (111,112)
	Atorvastatin 10mg / day for 10 days increased acetylcholine induced vasodilation vs. placebo (p<0.01) (double-blind, crossover). (109)		
Hypertension control	After 6 months of antihypertensive therapy FMD improved in 250 of 400 women (62.5%) from 6.8% to 13.9%. Fewer women with vs. without improvement in FMD had a cardiovascular event (6.0% vs. 21.3%; p=0.0001). (29)	Yes (113,114)	unclear (112)
ACE inhibitors	Quinapril improved coronary responses to acetylcholine after 6 months of therapy vs. placebo (double-blind, randomized, placebo-controlled). (115)	Yes (113,114)	unclear (111,112)
ARBs	After 12 months of candesartan the forearm blood flow response to acetylcholine was improved (p<0.05). (101)	Yes (113,114)	unclear (111,112)
Nebivolol	Nebivolol increased forearm blood flow by 91% (p<0.01). L-NMMA inhibited the increase in flow from nebivolol. L-arginine abolished the inhibition of nebivolol response by L-NMMA. (117)	Yes (118)	maybe (119)
Aspirin	Aspirin intravenously improved vascular resistance response to acetylcholine in patiens with coronary artery disease (p=0.002). (120)	Yes (121)	unknown
Remote Ischemic Preconditioning	Ischemia and reperfusion injury blunted the blood flow response to acetylcholine in controls (p=0.03), but the response was preserved by remote ischemic preconditioning (p=0.66). (97)	maybe (96)	unknown
	Ischemia and reperfusion injury reduced FMD (p<0.001), but not following remote ischemic preconditioning (p=NS). (98)		

Figure 6 Therapies that improve endothelial function and their effects on cardiovascular end points and ED. CV, cardiovascular; FMD, flow-mediated dilation; FDA, Food and Drug Administration; CHF, congestive heart failure; L-NMMA, N^G-monomethyl L-arginine; unknown, not extensively studied; unclear, results are indeterminate or conflicting; maybe, preliminary results show a benefit.[16,29,73,92–98,100–121]

begun. Because endothelial dysfunction occurs early in the disease process, some patients might not yet have developed atherosclerotic plaques visible on angiography. Other patients may have already developed vulnerable coronary plaques (~30% stenosis) that do not limit flow but are susceptible to rupture and myocardial infarction. Other patients may have advanced disease and flow-limiting stenoses. A full cardiovascular workup including stress testing, noninvasive imaging such as CT angiography and even coronary angiography may be needed to determine the degree of obstructive coronary artery disease.

Therapy for endothelial dysfunction

To date, the only class of medications that specifically target endothelial function is PDE5 inhibitors. NO decreases vascular (and trabecular) smooth muscle tone by increasing cGMP levels. PDE5 hydrolyzes cGMP, hence PDE5 inhibitors increase cGMP and enhance the effects of NO. PDE5 inhibitors are first-line therapy for ED. They have recently been approved by the Food and Drug Administration for the treatment of pulmonary hypertension. In clinical trials PDE5 inhibitors seem to benefit patients with congestive heart failure, especially those with secondary pulmonary hypertension.[92] In contrast to on-demand dosing, chronic daily dosing of PDE5 inhibitors provides marked, sustained improvement in peripheral endothelial function and has improved ED in some nonresponders to on-demand dosing.[93]

A variety of other medications, supplements and lifestyle habits have been shown to have a beneficial effect on endothelial function (Figure 6). The effect that these various interventions have on ED is still being investigated. Most of the medications that improve endothelial function also improve cardiovascular end points; however, antioxidants[94] and estrogens[95] have not shown cardiovascular benefit in large-scale analyses. As such, it is not known whether developing therapies targeted specifically to endothelial function will decrease major adverse cardiac events.

Ischemic preconditioning

Rather than inflict harm, 5 min of brachial artery occlusion protects endothelial cells, the heart and other organs through remote ischemic preconditioning.[96–98] Ischemic preconditioning is a phenomenon in which brief periods of nonlethal ischemia, followed by reperfusion, protects local tissue against later episodes of prolonged ischemia and reperfusion. In remote ischemic preconditioning, protection is conveyed to distant tissues or organs. A common ischemic preconditioning stimulus is blood pressure cuff inflation around the upper arm in a series of three 5-min inflations to 200 mm Hg with intervening 5-min periods of reperfusion while the cuff is deflated. More prolonged periods of ischemia ($\geqslant$20 min) and reperfusion cause local endothelial dysfunction and inhibit FMD and the vasodilatory response to acetylcholine.[97,98] In a series of studies, Kharbanda *et al.*[97] and Loukogeorgakis *et al.*[98] showed that a series of three 5-min cuff inflations preserved endothelial function in the contralateral brachial artery when exposed to acetylcholine or more prolonged ischemia (20 min) and reperfusion. In addition, using this remote ischemic preconditioning protocol before coronary artery bypass graft surgery in a randomized, placebo-controlled design, Hausenloy *et al.*[96] showed cardiac protection by lower levels of troponin T postoperatively in the preconditioning group versus the control group. Not only does remote ischemic preconditioning preserve endothelial function but it also seems to influence cardiac end points, perhaps through a mechanism involving endothelial cells.

Conclusion

The remarkable endothelial cell is vital to numerous biological processes, including inflammation, platelet aggregation, thrombosis and vascular smooth muscle proliferation, in addition to providing a selectively permeable barrier to blood and modulating vascular tone and blood flow. Endothelial dysfunction is a systemic process that occurs early in atherosclerosis and seems to contribute to the pathogenesis of atherosclerosis. There are numerous modalities to test endothelial function, including catheter-based methods, venous occlusion plethysmography, high-frequency ultrasound, peripheral arterial applanation tonometry, digital pulse amplitude tonometry, digital thermal monitoring, the L-arginine test and measuring compounds released from endothelial cells. The ideal clinical test to assist cardiac risk stratification and diagnose vasculogenic ED is closer to becoming realized. Various medications and lifestyle habits have already been shown to improve endothelial function, most of which also improve cardiovascular end points. The endothelial cell's pivotal location and marked influence on the cardiovascular system make it a promising target for future research and make it likely to become more important in clinical medicine.

Conflict of interest

Dr Kloner is on an advisory board to IC Therapeutics (Ischemic Conditioning Therapeutics), a company related to Endothelix. In the past, Dr Kloner has served as a speaker and consultant to Pfizer and Lilly.

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