Review article

Endothelial dysfunction and cardiovascular disease

R. Jay Widmer*, Amir Lerman

Division of Cardiovascular Diseases, Department of Internal Medicine, Mayo Clinic and College of Medicine, Rochester, MN 55905, USA

*Email: Widmer.Robert@mayo.edu

INTRODUCTION

The endothelium, by area alone, is one of the largest organs in the body comprised of up to trillion cells, weighing over 1 kg, and covering nearly 3 m² in a 70 kg male.[1,2] Moreover, it interacts with nearly every system in the body, and has been implicated in end organ diseases of systems such as neurologic, renal, hepatic, vascular, dermatologic, immunologic, and of course cardiac. The endothelium is a single vessel layer standing as the ultimate layer between blood and vascular supply to other tissues, and serving multiple purposes. The first and foremost is a hemostatic balance between thrombosis and anticoagulation via a careful series of checks and balances discussed below. Additionally, the endothelium regulates vascular tone, carefully balancing vasoconstriction and vasodilation to provide adequate perfusion pressure to target organs. Other functions include regulation of angiogenesis, wound healing, smooth muscle cell proliferation, fibrosis, and inflammation. Factors that adversely affect the endothelium include common cardiovascular risk factors such as tobacco use, obesity, age, hypertension, hyperlipidemia, physical inactivity, and poor dietary habits. Alternatively, factors that positively impact the function of the endothelium are, essentially, beneficial lifestyle habits opposite to those mentioned above such as increased physical activity, dietary habits which include anti-inflammatory and anti-oxidant foods, and some pharmaceutical agents such as L-Arginine.

Presently there is no guideline directive – either pro or con – to routinely include endothelial function testing in cardiovascular disease (CVD) risk assessment.[3−5]

ENDOTHELIAL PHYSIOLOGY

Under basal conditions, the endothelium functions to maintain the vessel in a relatively neutral state favoring dilatation over constriction. However, the endothelium has the capacity to respond to various intrinsic physical stimuli, such as shear stress, temperature, transmural pressure, and external stimuli such as temperature, mental stress, neurohumoral responses, and medications among others. The endothelial-dependent response to vasodilate is principally regulated in response to shear stress by a release of nitric oxide (NO) synthesized from the amino acid L-arginine by endothelial nitric oxide synthase (eNOS) which leads to the production of intracellular cyclic GMP.[6] In such a state when NO-mediated vasodilation is compromised, the vasodilatory response is thought to be facilitated by cytochrome-derived factors, naturetic peptide, and prostacyclin.

Dysfunctional endothelium is seen when there is an imbalance in NO production and consumption, favoring consumption and reduced production. Such a pathologic state creates favorable conditions for platelet plus leukocyte activation and adhesion, as well as the activation of cytokines that increase the permeability of the vessel wall to oxidized lipoproteins and inflammation mediators, finally resulting in structural damage of the arterial wall with smooth muscle cell proliferation and atherosclerotic plaque formation.

Endothelial dysfunction is usually ubiquitous throughout the body as patients with known atherosclerosis also have endothelial dysfunction in peripheral vascular beds that may not be affected by frank atherosclerosis. Endothelial dysfunction is also seen in patients with a family history of early

CVD and no other risk factors,[7] hypertriglyceridemia,[8] elevated LDL and reduced HDL cholesterol,[9] nicotine use,[10] obese patients with minimal coronary artery disease (CAD),[11] patients with insulin resistant,[12,13] patients with first degree relatives with DM2,[14] cardiac syndrome X,[19] elderly patients[15,16] irrespective of other comorbidities,[17] and mental stress[18,19] which is thought to be mediated through endothelin.[20] The progression of endothelial dysfunction is related to the intensity and duration of proven risk factors, and to the total risk of the individual subjects.[21,22]

The impact of endothelial dysfunction typically manifests in frank atherosclerosis requiring either percutaneous or surgical revascularization. Data from our group has demonstrated that in a multi-center study, peripheral endothelial dysfunction is seen in nearly 75% of patients following PCI [Widmer, RJ, et al. 2014. *In Review*]. Endothelial dysfunction appears to result from reduced levels of NO bioavailability,[23] and largely related to baseline risk factors, thus making it an attractive alternative metric to monitor for secondary prevention.[24] Furthermore, peripheral endothelial dysfunction in patients following PCI is a known predictor of restenosis.[25] Surgical revascularization is also associated with endothelial dysfunction and can be assessed properly by reactive hyperemia or laser Doppler.[26] Patients who recently underwent CABG have reduced endothelial function largely related to poor NO bioavailability and poor glycemic control.[27]

Other cardiovascular entities can also cause reduced endothelial function as microvascular disease is seen in patients with cardiac amyloidosis.[28] Moreover, endothelial dysfunction can serve as a prognostic indicator in children with familial cardiomyopathies.[29] Finally, myocardial bridging is closely associated with coronary endothelial dysfunction which can be detected by fractional flow reserve.[30]

Abnormal endothelial function is attributed to high oxidative stress and inflammation – both processes lead to abnormal NO metabolism (bioavailability, use/response, production, release, and degradation), which can be exacerbated with other conditions (cold, mental stress, anger) that are known to produce a global vasoconstriction. Increased oxidative stress is characterized by a measurable increase in reactive oxygen species (ROS) which can result from impaired NO synthase, decreased L-arg uptake, increased oxidized LDL cholesterol (Ox-LDL),[31] or reduced superoxide dismutase (SOD) an enzyme pivotal in the clearing of ROS.[32] Hyperlipidemia is known to increase ROS which will reduce the bioavailability of NO, yet can be ameliorated with correction of hyperlipidemia.[33] Another potential contributor to impaired NO bioavailability is a decrease in tetrahydrobiopterin (THB).[34,35] Furthermore, replenishing THB stores appears to improve endothelial dysfunction, even in hyperlipidemic patients.[36] Another potential contributor to endothelial dysfunction is elevated asymmetric dimethylarginine (ADMA) which is an endogenous competitive inhibitor of NO.[37,38] ADMA has been linked to reduced endothelial function as well as erectile dysfunction in patients at moderate risk for CVD.[39] Furthermore, Ox LDL can increase ADMA further compounding known risk factors in patients with CAD[4,30] even leading to increased events in those found to have elevated levels of ADMA.[40] Finally, low-flow vascular states such as reduced cardiac output which reduces endothelial shear stress in conditions such as heart failure – possibly from reduced L-arginine,[41] or with other vascular injury or occlusion can contribute to an alteration in bioavailable NO and varying endothelial function.[42]

Localized infection, particularly in the oral cavity, leading to increased systemic inflammation and impaired endothelial function has been postulated to be a contributor to CVD. Chlamydia pneumonia infections[43] and immediate systemic inflammatory states which would mirror sepsis[44] have been shown to increase global inflammatory markers and endothelial dysfunction. Furthermore, H. pylori positive patients have reduced endothelial function ameliorated by treatment.[45] However, global treatments with antibiotics in high risk patients show no benefit on CVD outcomes.[46,47]

External beam radiation[48] and chemotherapy (particularly doxorubicin and daunorubicin[49]) have been shown to reduce endothelial function in patients independent of other risk factors presumably through endothelial cell death and reduced NO availability.

Endothelial dysfunction is also associated with the development of transplant vasculopathy. In a study of 73 patients who underwent heart transplantation, the presence of endothelial dysfunction predicted the development of clinical end points, including angiographic vasculopathy or cardiac death (graft failure or sudden death).[50]

- The endothelium makes up one of the largest organ systems in the body by surface area.
- Normal endothelial function allows for the symbiotic balance between vasoconstrictive and vasodilatory (namely via NO) stimuli to allow adequate end organ blood perfusion.

- A disruption in normal endothelial physiology through a number of various mechanisms causes endothelial dysfunction – a progenitor toward atherosclerosis and a predictor of future cardiovascular events.

ENDOTHELIAL DYSFUNCTION PREVALENCE

The true prevalence of peripheral endothelial function worldwide is not fully known as we only have samples of larger studies which assess peripheral endothelial function in different methods and without specific guidelines regarding cut-off values for which a patient's endothelium is considered dysfunctional. Older observations demonstrate that nearly 60% of community-dwelling participants who undergo coronary angiography and are found to have non-obstructive CAD.[51]

Similarly, the WISE study found that roughly 50% of those undergoing clinically indicated coronary angiography, but without obstructive disease were found to have coronary endothelial dysfunction.[52] Peripheral endothelial dysfunction, depending on the defined cutoff value defining such, can be found in up to 75% in those presenting with acute coronary syndrome (ACS) and undergoing percutaneous coronary intervention (PCI) for such [Widmer RJ, et al. 2014. *In Review*].

Other systemic disorders known to be characterized by endothelial dysfunction include transplant vasculopathy,[50] autoinflammatory diseases,[53] gastrointestinal disorders such as celiac disease and irritable bowel syndromes,[54,55] hematologic/oncologic survivors,[56] cryptogenic stroke,[57] and neurocognitive disorders.[58]

- Currently there is a wide range of reported prevalence of both coronary and peripheral endothelial dysfunction secondary to the heterogeneity of the studies examining the phenomenon and arbitrary cutoff values.
- Endothelial dysfunction is prevalent in CVD, however can also be found in patients with neurologic, gastrointestinal, rheumatologic, hematologic, and renal diseases.

TYPES OF ENDOTHELIAL FUNCTION TESTING

The quantification of endothelial health has commonly been divided into peripheral endothelial function – a systemic measure of endothelial function – versus coronary endothelial function, which must be assessed with invasive angiography. Testing involves pharmacological and/or physiological stimulation of the endothelial release of NO and other vasoactive substances. All the techniques have in common that they measure the response of the vessels to endothelial-dependent stimuli, mainly reactive hyperemia (shear stress) or vasoactive substances. Indeed, both macrovascular endothelial dysfunction, as measured by flow-mediated dilation,[59,60] and microvascular endothelial dysfunction,[61,62] have been found to be independent predictors of future cardiovascular events in large cohort studies in healthy individuals over and above traditional risk factor assessment. Endothelial function testing modalities have also been found to correlate with other novel cardiovascular testing modalities such as coronary calcium scoring.[63,64]

Invasive endothelial function testing (coronary)

Quantitative coronary angiography can be used to directly and invasively examine the change in diameter in response to intracoronary infusions of endothelium-dependent vasodilators such as acetylcholine.[65,66] In healthy coronary epicardial and microvascular vessels, acetylcholine generates an NO-mediated vasodilatory response quantitatively assessed by angiography or intravascular ultrasound (IVUS). In patients with endothelial dysfunction, this effect is blunted or vasoconstriction is paradoxical. Endothelial function of the coronary microvasculature can be assessed with intracoronary Doppler techniques to measure coronary blood flow in response to pharmacological (usually intracoronary adenosine or nitroprusside) or physiological stimuli.

Noninvasive tests for assessment of coronary endothelial function include Doppler echocardiography, positron emission tomography, and phase-contrast magnetic resonance imaging. In patients without obstructive coronary artery disease but impaired coronary vasodilatory capacity in the face of a vasodilator challenge, there is a marked increase in CVD events over the subsequent two years.[66] This notion was furthered in a similar experiment following patients for nearly 8 years showing a 20–40% reduction in survival over the subsequent 7.7 years depending on their coronary vascular reactivity to any certain number of stimuli.[67]

Invasive coronary vasoactive testing can also be important in patients with known CAD as a sub-study of the COURAGE trial evaluated the fractional flow reserve (FFR) to differentiate between coronary lesions causing ischemia, and blockages not causing ischemia.[68] The study showed that patients with ischemic blockages who were treated with revascularization had a long-term significant improvement in comparison with those who were only given medical intervention. In another multicenter study, patients with multi-vessel coronary artery diseases who underwent invasive interventions (PCI) with drug-eluting stents that were guided by FFR results had a significant reduction in the rate of the composite end-point of death, myocardial infarction, and target vessel revascularization at one year post-intervention.[69] Another example of the efficacy of the FFR test can be seen in a study that showed an almost 43% potential reduction in the need for bypass surgery in patients who underwent FFR after having a coronary angiogram.[70] It is therefore very important to examine the individual contribution to the physiology of an ischemic lesion, and not just its anatomy.

A similar method, but based on slightly different physical properties, by which to test coronary microcirculatory function deals with thermodilition and index of microcirculatory resistance (IMR). This technique is similar to pharmacological and pressure-based techniques, but in this case using intracoronary temperature measurements to approximate flow.[71] This has been shown to be independent of epicardial vascular function, reproducible, and has even been evaluated in STEMI patients, providing important prognostic information regarding ventricular function at three months.[72]

Non-invasive endothelial function testing (arterial)

Brachial artery ultrasound is a commonly used and widely accepted measure of peripheral macrovascular endothelial function.[73] In this test, inflating a blood pressure cuff at suprasystolic pressures for 5 minutes occludes the upper arm proximal to the ultrasound measurement. Upon the release of the occlusion, an increase in shear stress results in an endothelial-dependent, NO-driven, flow-mediated dilation (FMD) of the brachial artery. Both diameter and blood velocity are assessed before and after occlusion with results being reported as a percent change from baseline. These measurements should be made at the end of diastole. The reported vascular response to increased flow has been shown to be a surrogate for measuring coronary endothelial function.[74]

Aside from reactive hyperemia, stimuli for measuring endothelial reactivity can include exercise, mental stress, or sympathetic nervous activation through the cold pressor test. As will all vascular reactivity tests, brachial artery ultrasound measurements can be potentially confounded by such conditions such as the amount, type, and time after food consumption; medications; exercise; ambient temperature; menstrual cycle stage; type of machinery and equipment; and variations in the protocol between subjects or experiments (supine, dark room, thermo-neutral settings). Furthermore, occlusions made too proximal can exacerbate the FMD response creating a potential for false negative results.[75]

Observational data from the MESA study demonstrates that peripheral endothelial dysfunction as measured by FMD of the brachial artery is associated with a higher rate of incident adverse CVD events during a five-year follow-up period.[59] FMD has been linked with increased CVD risk in those patients with known CVD risk factors.[76] Furthermore, data from a recent meta-analysis provides evidence that FMD could be used as an independent prognostic indicator of future CVD events and offers incremental risk factor stratification in addition to traditional risk factors.[77] While some have recently argued that there is only minimal data that FMD adds to risk stratification,[78] a more overwhelming body of evidence argues for the use of FMD measurements to provide important additional CVD risk factor stratification and or response to therapies.[79,80]

Impedance plethysmography is a method of assessing endothelial function via strain-gauge venous impedance plethysmography which examines the changes in forearm blood flow in response to direct intravascular administration of vascular agonists.[81] Due to the invasive nature of this test it is primarily used in research settings, and rarely utilized clinically (Figures 1,2).

Low-flow-mediated constriction (L-FMC) quantitates the reaction in forearm conduit artery diameter occurring in response to a reduction in blood flow, and resultantly, shear stress.[82] This method is similar to FMD measurements of the brachial artery, and is often used in concert to gain additional information regarding arterial reactivity[83] as L-FMC is a better measure of resting, basal arterial tone and thought to only be partially NO-mediated.[84] Exact mechanisms of this reaction are still being elucidated, and it is becoming increasingly important as the rate of radial interventional approaches increases.[42] Nevertheless, the combination of these two measurements has been shown to be closely

Coronary vasoreactivity and atherosclerotic disease progression.

Baseline   **Acetylcholine**

Nitroglycerin   **Follow up (3.7 years)**
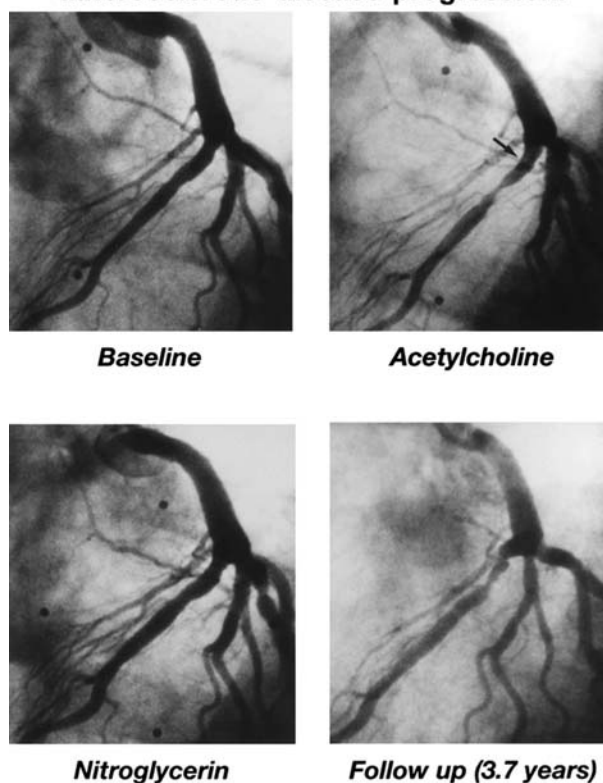
Schächinger V et al. Circulation. 2000;101:1899-1906

Figure 1. Coronary vasoreactivity and atherosclerotic disease progression. Top left shows baseline coronary angiogram of a patient in whom focal paradoxical vasoconstriction to acetylcholine occurred in the proximal left anterior descending artery (top right) at the time of the initial vasomotor testing (arrow indicates tip of acetylcholine infusion catheter). Injection of nitroglycerin (bottom left) demonstrates only minimal vasodilation and unmasks an atherosclerotic plaque at the site of paradoxical vasoconstriction to acetylcholine. During follow-up 3.7 years later, the patient was admitted to the hospital with an acute coronary syndrome. Coronary angiography revealed focal progression of atherosclerotic disease (bottom right) at the site of the initial paradoxical vasoconstriction to acetylcholine.

correlated to the severity of CAD,[85] and even provide additive risk factor stratification to traditional risk factors.[86,87]

Peripheral arterial tonometry (PAT) is a technique commonly used to assess microvascular endothelial function via changes in finger pulse wave amplitude in response to reactive hyperemia.[88–91] Testing for endothelial function involves the inflation of a blood pressure cuff to supra-systolic pressures. During this process there are two PAT probes connected to the fingers in both arms. The probe that is connected to the arm where the blood pressure cuff is inflated for 5 minutes is used to assess the reactive hyperemic response, a surrogate and a marker for endothelial function. These methodologies are non-invasive, are designed to eliminate environmental interference, and are independent of the subject's knowledge and conscious control of signals generated.[88,90] The RH-PAT index is defined as the ratio of the average pulsatile blood volume response, at timed intervals after deflation, to the baseline pulsatile blood volume response; i.e. the average amplitude of the RH-PAT signal over 60 seconds at 1, 2, 3 and 4 minutes after cuff deflation divided by the average amplitude of the RH-PAT signal over 3.5 minutes prior to cuff inflation (during baseline equilibration). A diagram of the PAT device is shown in Figure 3.

Recent work has shown a correlation between endothelial function as measured through PAT, and the accepted standard of invasive assessment of endothelial dysfunction of the coronary arteries.[92] Moreover, Goor et al. has demonstrated that there is a characteristic PAT signal response to mental stress, with diminution of the signal amplitude during stress.[90] However, this test is only thought to be partially dependent on NO,[93] with other factors such as the sympathetic nervous system thought to

Typical example of ultrasound scanning of brachial artery of a female subject in the F phase at rest (A) and during reactive hyperemia (B).

A. Rest

B. Reactive Hyperemia

a

p

a

p

5 mm
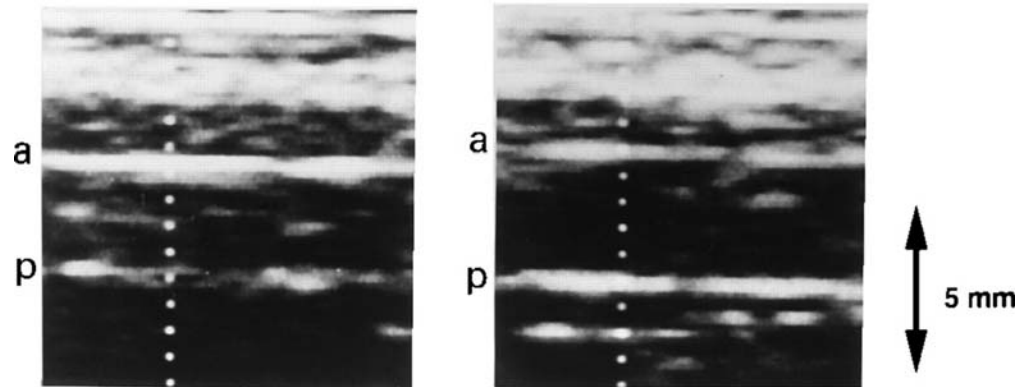
Hashimoto M et al. Circulation. 1995;92:3431-3435

Figure 2. Typical example of ultrasound scanning of brachial artery of a female subject in the F phase at rest (A) and during reactive hyperemia (B). The diameter of the artery increased from 3.1 mm (A, rest) to 3.8 mm (B, reactive hyperemia) in response to increased blood flow. a indicates anterior wall of the brachial artery; p, posterior wall of the brachial artery.

affect the microvascular response to certain stimuli.[94] There are discordant reports as to the agreement between FMD and PAT as some reports highlight a discordance with PAT and FMD measurements,[95,96] while others find an association between the two tests.[94] There still appears to be a strong contribution by NO to both physiologic responses leaving mechanistic work to resolve these discrepancies yet to be finished.[97]

In addition to the endothelial function test being a predictive parameter for coronary disease onset, it can also predict the effectiveness of a treatment given to patients with cardiovascular disease. One study followed a group of hypertensive women without significant heart disease, and who followed a similar antihypertensive regimen. While all women had similar reductions in blood pressure, the individuals whose endothelial function improved had half as many cardiovascular events compared to those women who showed no improvement in endothelial function.[98] Similarly, a high-risk group of patients with significant coronary diseases were treated with optimal medical therapy and given standard medications prescribed for their disease. The patients underwent endothelial function tests at baseline and six months with improvement seen in 50% of the patients. Those with improved endothelial function had fewer cardiovascular events during the follow-up period whereas the group that did not have improved endothelial function had an increased CVD event rate.[99]

Additional observational data examining microvascular endothelial function with PAT demonstrates people with relatively normal risk factors, but reduced endothelial function, had a higher incidence of heart disease, hospitalization, and death after seven years of follow-up as compared to those without
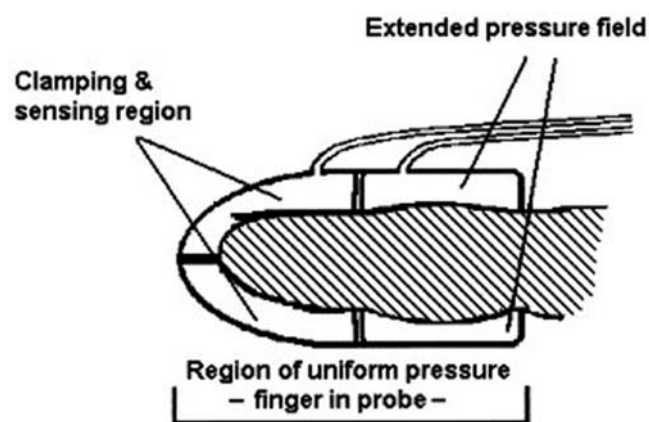
Extended pressure field

Clamping & sensing region

Region of uniform pressure
– finger in probe –

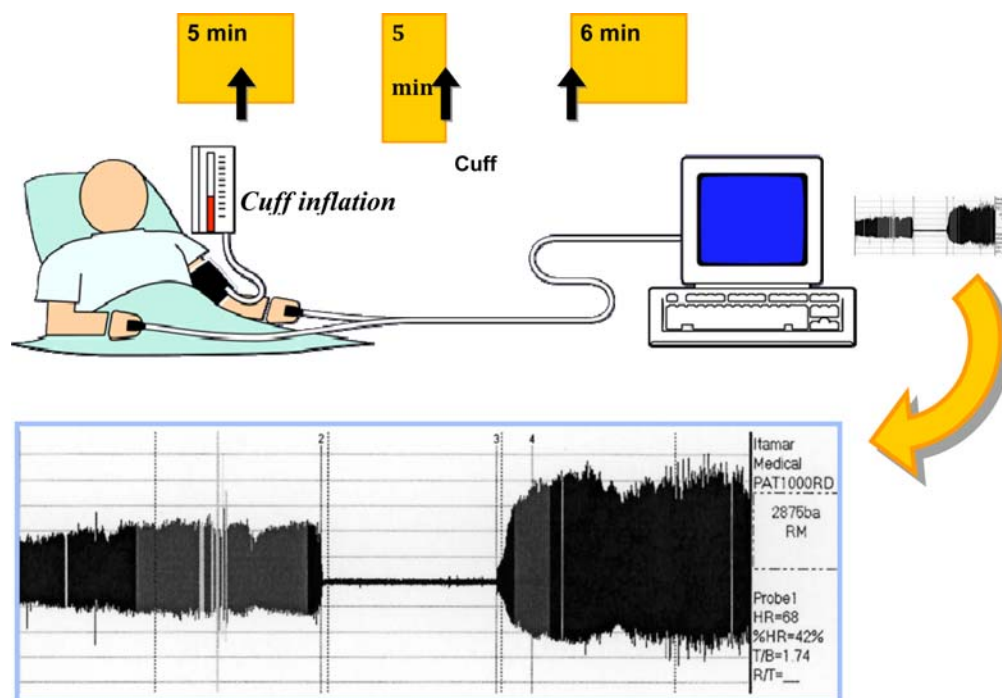**Figure 3A.** Diagram of PAT device on digit.

Figure 3B. Diagram of RH-PAT protocol set-up.

endothelial dysfunction.[100] Intuitively, those with a high Framingham risk score and endothelial dysfunction were at the greatest risk, followed by those with a normal Framingham score but with endothelial dysfunction, and then those with a high Framingham score but with normal endothelial function.[100] Finally, those patients already with obstructive CAD, PAT can predict future adverse CVD outcomes.[99]

Other non-invasive measures of endothelial function

Measures of venous endothelial function are used as the pathogenesis of arterial and venous clots share the characteristic of endothelial dysfunction in conditions favorable for generation and as 70% of the circulating blood is contained within the venous system. However, this is seldom used clinically, as the techniques typically involve difficult measures that lack reproducibility such as the dorsal vein technique and radionuclide venous plethysmography. Thus, as patients who will have venous endothelial dysfunction typically have arterial endothelial dysfunction, we suggest using methods mentioned above (Figures 4 – 6).

Traditional imaging-based modalities have been clinically utilized and verified to assess microvascular function. Myocardial PET imaging has been tested using a cold pressor test in patients with diabetes,[101] and verified in a larger cohort of patients.[102] Furthermore, cardiac magnetic resonance perfusion has been correlated with endothelial dysfunction in patients without overt coronary artery disease[103] as well as those with typical angina but relatively normal angiograms.[104] However cost and limited availability of the resources necessary for such prevent widespread adoption of these modalities. Other measures of endothelial function are currently in various stages of use and design – mainly in the research realm. Measures of endothelial progenitor cells (EPCs) and subtypes which assist in endothelial repair and function have been associated with Framingham scores[45] and various measures of CVD.[46] Statins,[48] erythropoeitin,[49] and exercise[50] all increase EPCs numbers and function. However, the exact subpopulation of beneficial EPC subtypes remains to be defined.

Genome wide array sequencing (GWAS), single nucleotide polymorphisms (SNPs), microRNAs (miRs), and even some urinary biomarkers have all been linked with various stages of endothelial dysfunction and early atherosclerosis, however these are not yet in clinical use (Table 1).

Urine microalbuminuria[21] and others have also been studied as markers of endothelial function, however they are also not in clinical use.

Impaired coronary artery vasoreactivity predicts CV events

Flow-dependent dilation

≥19.0 percent

p = 0.004

10.3 – <19.0 percent

p = 0.025

p = 0.004

≤10.3 percent

Proportion without cardiovascular event, percent

Months

Endothelium-independent vasodilation

≥42.6 percent

p = 0.007

22.9 – <42.6 percent

p = 0.030

≤22.9 percent

p = 0.006

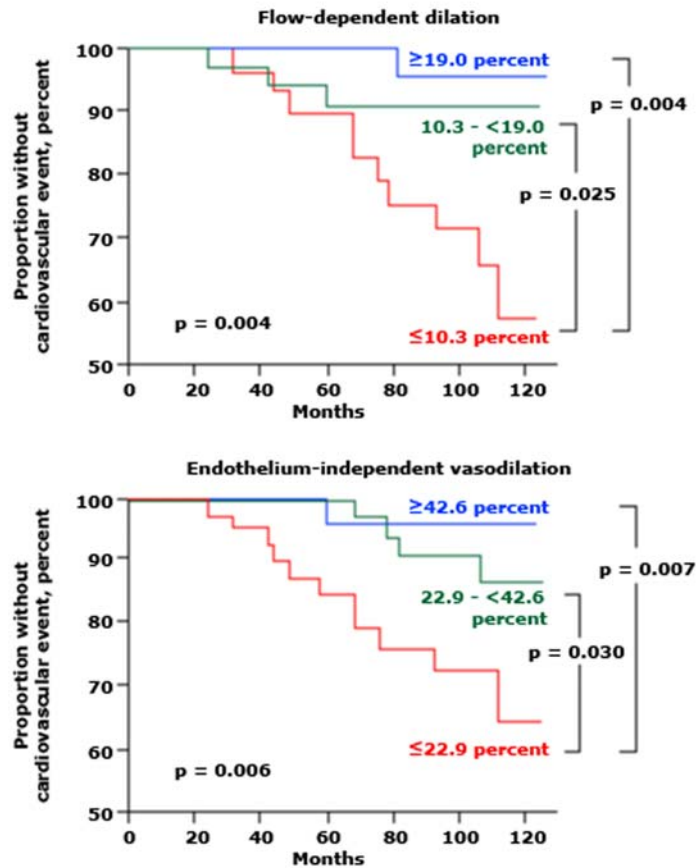Proportion without cardiovascular event, percent

Months

Figure 4A. Coronary artery vasoreactivity was evalauted in 147 patients with documented coronary heart disease (CHD) or risk factors for CHD; flow-dependent dilation, assessed with intracoronary papaverine or adenosine (upper panel), and flow-independent vasodilation, induced by nitroglycerin (lower panel), were divided into tertiles (percent arterial dilation). During a median follow-up of 6.7 years, impaired vascular reactivity was associated with a significantly higher cardiovascular event rate (cardiovascular death, unstable angina, myocardial infarction, revascularization, stroke, peripheral artery revascularization); the incidence of events was highest in those with the least dilation. *Data from Schachinger V, Britten MB, Zeiher AM. Circulation 2000; 101:1899.*

- Endothelial function can be tested in a variety of ways measuring both macrovascular endothelial function with brachial artery ultrasound as well as microvascular endothelial function with peripheral arterial tonometry.
- Macrovascular endothelial function is typically measured by flow-mediated dilation using ultrasound of the brachial artery measuring vascular reactivity to such stimuli as medications and reactive hyperemia.
- Low-flow-mediated constriction is a newer method of testing macrovascular function specifically focusing on the resting state of the endothelium in a non-NO-dependent manner.
- Microvascular endothelial function measures vascular reactivity to the same stimuli, however typically utilizing peripheral tonometry or pulse wave amplitude to approximate blood flow.
- There are no guideline-based recommendations to use endothelial function testing clinically, however, such tests can augment combined risk factor assessments in patients who may fall between two risk factor categories.
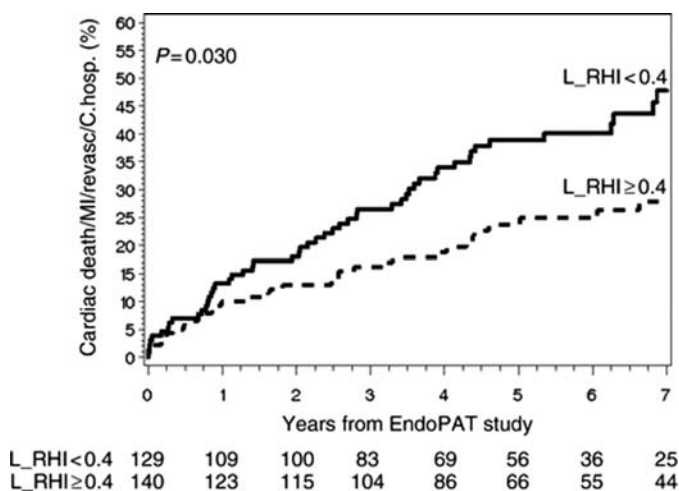
Figure 4B. Cardiovascular adverse events: a combination of cardiac death/myocardial infarction/coronary revascularization and cardiac hospitalizations in patients with and without low L_RHI ($<$0.4).

Preoperative Risk Assessment – Assessment of endothelial function by brachial ultrasound has been used for preoperative risk stratification in patients undergoing vascular surgery.[41] An FMD measurement for the purpose of perioperative risk stratification was assessed in a study of 187 patients undergoing high-risk (vascular) surgery. Low flow-mediated dilation was an independent predictor of a cardiac event at 30 days (odds ratio 9)[41] and of late events at 1.2-year follow-up.[105]

ENDOTHELIAL DYSFUNCTION TREATMENTS/THERAPIES

1. *Life style modifications (diet, exercise, smoking, weight reduction . . .)*

Mediterranean diet – A low fat diet is usually the first step in treating hypercholesterolemia. A Mediterranean diet reduces serum LDL-cholesterol and lowers the risk of cardiovascular events in patients with a myocardial infarction. These benefits are associated with an improvement in endothelial function.[123,124] These findings have been confirmed in a larger randomized controlled trial showing improved endothelial function in participants who adhered to the Mediterranean Diet and exercise.[125]

Other Dietary Interventions – Recently, there has been a plethora of trail data showing that substances such as dark chocolate,[126] nuts,[127] olive oil,[128] plant-based foods,[129] green tea,[130] and alcohol consumption[131] have all shown to be beneficial to improving peripheral endothelial function. Indeed, proper dietary measures should serve as a cornerstone toward improving endothelial function.

Aerobic exercise and weight loss – Regular aerobic exercise is associated with a reduced risk of cardiovascular events, especially in middle-aged and older adults; it also can modify many of the traditional risk factors for coronary disease, including endothelial dysfunction. Although there has been some debate over the initial endothelial response to exercise, the overall data has

Table 1. Factors that cause and interventions that improve endothelial dysfunction

Factors associated with endothelial dysfunction	Interventions that improve endothelial function
Increased age	L-arginine
Male sex	Antioxidants
Family history of CHD	Smoking cessation
Smoking	Cholesterol lowering
Increased serum cholesterol	ACE inhibitors
Low serum HDL-cholesterol	Exercise
Hypertension	Mediterranean Diet
Increased serum homocysteine	
Diabetes mellitus	
Obesity	
High-fat meal	

been positive toward this lifestyle behavior and endothelial function. As an example, one study of 68 healthy men, aged 22 to 35 and 50 to 76, who were either sedentary or endurance-exercise trained, found that endurance-trained men did not have an age-associated decline of endothelium-dependent vasodilation; in addition, regular aerobic exercise restored the loss of endothelium-dependent vasodilation in previously sedentary middle-aged and older healthy men.[132] Weight loss alone has been shown to be beneficial to endothelial function.[133] Exercise has also been seen to improve endothelial function in such high risk patients as those with peripheral vascular disease[134] and heart failure.[135]

Similarly, obesity has been linked with endothelial dysfunction,[136] and conversely weight loss is associated with a significant reduction in cytokine levels and an improvement in endothelial dysfunction.[137,138]

2. **NO pathway (L-arginine, PDE-I)**

L-arginine – The intravenous or intracoronary administration of L-arginine, the physiologic precursor for NO, can acutely improve endothelium-dependent, but not endothelium-independent, vasodilation in patients with hypercholesterolemia or coronary atherosclerosis.[92] L-arginine has been tested in multiple smaller, randomized, clinic trials, and shown to have benefits; however, the stereoisomer, D-arginine, is ineffective.[93] Additionally, and small randomized trial consisting of 30 patients appeared to show no benefit on multiple measures of endothelial health such as NO bioavailability, cell adhesion molecules, or brachial artery FMD with 9 g daily of L-arginine supplementation.[94] These patients, however, were on optimal medical therapy for CVD prevention.

In contrast, a similarly-sized trial showed that a similar dose of L-arginine after 6 months had a significant improvement in patients coronary endothelial function and resultantly improved anginal symptoms.[95] Additional work has shown short-term L-arginine supplementation to be of clinical benefit in a randomized study of 36 patients with stable class II and III angina. Compared to placebo, two weeks of therapy with a medical food bar enriched with L-arginine improved flow-mediated vasodilation, treadmill exercise time, and quality of life scores.[96]

The most recent data regarding L-arginine and endothelial function appears to show that when given at doses of 2 g thrice daily for one month there is reduced blood pressure and angina symptoms in concert with improved endothelial function and quality of life in hypertensive patients without obstructive CAD.[97] Thus, although a narrow clinical niche, and a less-than-convenient dosing regimen, L-arginine supplementation can be beneficial in patients with non-obstructive CAD and debilitating angina by improving CVD risk factor parameters and symptoms.

The longer-term effects of oral L-arginine have also been evaluated. Among patients with heart failure, oral L-arginine improved endothelial function, arterial compliance, and functional status.[98]

The potential benefits associated with L-arginine therapies are presumably mediated by increased NO activity, particularly as it applies to improving the bioavailability of NO in areas of reduced endothelial shear stress.[99] In addition to improved endothelial function, L-arginine supplementation has also been implicated in reducing plasma endothelin levels,[95] reduced symptomatic burden via apoptosis of proliferating vascular smooth muscle cells leading to atherosclerotic plaque regression other changes that have been described include lower plasma endothelin concentrations,[100] and finally arresting atherosclerotic plaque development in an animal model.[101]

Phosphodiesterase inhibitors such as sildenafil have also been shown to be beneficial to improving peripheral endothelial function in a small cohort of diabetic men,[106] as well as enhance penile blood flow and erectile function.[107] Larger-scale data, however, does not exist, and these agents have recently been found to be of no CVD benefit in patients with heart failure and preserved endothelial function.[108]

Nicorandil – This anti-anginal, unavailable in the US, has dual nitrate and potassium-ATP channel agonist properties by increasing the formation of cyclic GMP. This agent is effective in improving endothelial function in patients without prior coronary artery disease after one year with concomitant reductions in inflammatory markers.[109] Despite its theoretical promise, there have only been limited data published, albeit positive, regarding the efficacy of nicorandil in patients ($n = 65$) with chronic stable angina as well as induced plaque regression in animal and human studies.[110]

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Widmer and Lerman. Global Cardiology Science and Practice 2014:43

3. *Channel pathways (Ca, K.)*

Nifedipine may have antioxidant effects and effects on endothelial nitric oxide synthase expression and activity. In a study of 454 patients undergoing percutaneous coronary intervention, endothelium dependent vasodilatation was assessed with intracoronary acetylcholine after six months of therapy with nifedipine, showing an improvement in endothelial function but no plaque regression.[111]

Ranolazine – This sodium channel inhibitor used for patients with refractory angina has been shown to alleviate symptoms of microvascular angina pain, however there was no significant change seen in microvascular function.[112] Furthermore, there has been improvement in endothelial function in smaller RCTs examining diabetic patients,[113] as well as patients with chronic stable angina.[114]

Supplementation of *taurine*, a semi-essential amino acid has also been shown to be marginally beneficial to endothelial function.[115]

4. *Receptor and enzyme pathways (beta-blockers, ET, ACE-I, ARB)*

Lipid lowering – Some but not all studies have found that endothelial dysfunction can be ameliorated or even eliminated with the use of a statin, and theoretically these medications will already be a mainstay in reducing CVD risk in most patients due to their lipid-lowering and anti-inflammatory mechanisms.[116] The combination of ACE-inhibition and statin therapy has also been shown to improve endothelial-dependent relaxation of the coronary vasculature through NO-dependent mechanisms.[117]

Fibrate therapy also improves fasting and postprandial endothelial function in patients with type 2 diabetes, as does omega-3 fatty acid supplementation.[118] The mechanism for this may be an increase in high density lipoproteins (HDL) and an attenuation of postprandial lipemia and the associated oxidative stress.[119] HDL lowering or niacin therapy appears to have no beneficial effect on endothelial health.[120]

Blockade of the renin-angiotensin system – Angiotensin converting enzyme (ACE) inhibitors may improve endothelial dysfunction, but this benefit may not be seen in all drugs in this class. In one report, quinapril, which has high tissue specificity for ACE improved endothelial dysfunction in patients with coronary disease.[121] The efficacy of quinapril was also evaluated in the TREND trial of men with coronary disease but without heart failure, hypertension, or lipid abnormalities improving endothelial dysfunction at six months.[122] These benefits were thought to be secondary to an improved NO-bioavailability through reduced bradykinin breakdown as well as improved ROS scavenging. As stated earlier, ACE-inhibitors do improve coronary endothelial resistance through NO-dependent mechanisms.[117]

Most studies have shown an improvement in endothelial dysfunction following the administration of an angiotensin II receptor blocker in patients with atherosclerosis or diabetes.[123–125] Furthermore, ARBs have been shown to improve coronary endothelial dysfunction,[126] and there is increasing evidence that direct renin inhibition improves endothelial function in at risk patients.[127]

Nebivolol, as there appears to be some increase in NO bioavailability with this beta-blocker.[128]

Aspirin – Studies suggest that aspirin improves endothelial dysfunction in patients with known atherosclerosis, likely through inhibition of cyclooxygenase-dependent vasoconstriction.[129] This can result in vasodilation and reduction in thrombosis, providing a potential mechanism for the beneficial effects of aspirin in atherosclerosis. However, most patients with endothelial dysfunction will already be on such a therapy, so the additive benefit is not likely to be substantial.

N-acetylcysteine (NAC), a thiol, is a pharmacologic precursor of L-cysteine. It augments the bioavailability of NO and can improve scavenging of ROS. One study of 16 patients with atherosclerosis found that NAC supplements improved coronary and peripheral endothelium-dependent vasodilation; the response to nitroglycerin was not affected, while the response to nitroprusside was potentiated only in the coronary arteries.[130]

Estrogen and other selective estrogen receptor modulators – Reports of estrogen therapy improving endothelial function in post-menopausal women[131] appear to have biologic plausibility as endothelial cells have estrogen receptors,[132] as well as through improved NO bioavailability[133] or through a reduction in coronary endothelin-1 levels.[134]

Similarly Tamoxifen and raloxifene are selective estrogen receptor modulators, having estrogen-like activity, and also found to have positive effects on FMD.[135] However, consideration for

the use of any of these hormonal therapies should be carefully evaluated in terms of global risks and benefits for the patient.

Testosterone may improve endothelial dysfunction, however the data is only in the form of a case series showing increased coronary artery vasodilatation.[136] Again, however, the overall risk of testosterone supplementation appears to outweigh the benefit of augmenting endothelial function with such, and we would recommend against this therapeutic option.

Endothelin receptor blockers – Elevated levels of endothelin is thought to play a role in endothelial dysfunction seen in heart failure and hypertension and the transient dysfunction induced by mental stress. A recent randomized, double-blind, placebo-controlled trial in patients at high risk for CVD showed a significant improvement in coronary microvascular endothelial function.[137] This therapy is still being researched for widespread use in targeted populations.

Insulin sensitizers – As diabetes and endothelial dysfunction are typically concomitant pre-atherosclerotic conditions, there is a body of literature detailing conflicting reports of the benefit of insulin sensitizers on endothelial function. Metformin is generally thoughts to improve peripheral endothelial function as evidenced by smaller RCTs in diabetic patients[81] and those with metabolic syndrome.[138] Extending these results, both metformin and rosiglitazone have been found to improve endothelial function in women afflicted with PCOS, however confounding effects of reductions in testosterone and HOMA results as well as normalization of meunstral cycles have left this debate unresolved.[139] Rosiglitazone has been found to attenuate impaired vasodilation in diabetic patients subjected to fatty acid meal challenges.[140] Conversely these results were not mechanistically validated as it was pioglitazone, not rosiglitazone, which reduced pharmacologically induced vasoconstriction in internal mammary artery grafts from diabetic patients.[141] Ultimately, these agents likely improve endothelial function in patients with diabetes; however, the multiple confounders present in these studies leave room for further work and research regarding their effect on endothelial function and CVD outcomes in larger RCTs.

Prognosis after therapy – A study in patients with coronary artery disease showed that persistent impairment of endothelial vasomotor function despite optimized therapy to reduce risk factors has an adverse impact on clinical outcome.[99]

SUMMARY AND RECOMMENDATIONS

- Currently, there are no FDA-approved treatments for endothelial dysfunction, as the treatment should encompass addressing the underlying comorbidity that lead to endothelial dysfunction.
- L-arginine, in large quantities (9 – 18 g daily), has been shown to have beneficial effects on both vascular reactivity and relief of symptoms from coronary endothelial function.
- ASA, statins, ACEI have all shown benefit in reducing CVD risk with endothelial function improvement likely to be a concomitant factor.
- Diet and exercise have both been shown to improve vascular reactivity, and should be encouraged as part of lifestyle behaviors beneficial toward overall CVD health.

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