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The Human Microcirculation – Regulation of Flow and Beyond

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Abstract

The microcirculation is responsible for orchestrating adjustments in vascular tone to match local tissue perfusion with oxygen demand. Beyond this metabolic dilation, the microvasculature plays a critical role in modulating vascular tone by endothelial release of an unusually diverse family of compounds including nitric oxide, other reactive oxygen species, and arachidonic acid metabolites. Animal models have provided excellent insight into mechanisms of vasoregulation in health and disease. However there are unique aspects of the human microcirculation that serve as the focus of this review. The concept is put forth that vasculo-parenchymal communication is multimodal, with vascular release of nitric oxide eliciting dilation and preserving normal parenchymal function by inhibiting inflammation and proliferation. Likewise, in disease or stress, endothelial release of ROS both mediates dilation and parenchymal inflammation leading to cellular dysfunction, thrombosis, and fibrosis. Some pathways responsible for this stress-induced shift in mediator of vasodilation are proposed. This paradigm may help explain why microvascular dysfunction is such a powerful predictor of cardiovascular events, and help identify new approaches to treatment and prevention.

Keywords

vasodilation; oxidative stress; microvascular dysfunction; endothelial shear stress; vascular smooth muscle

Subject Terms

Endothelium/Vascular Type/Nitric Oxide; Coronary Circulation; Oxidant Stress; Coronary Artery Disease; Vascular Disease

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“It has long been an axiom of mine that the little things are infinitely the most important” Sir Arthur Conan Doyle

Tasked with providing nutrients and removing metabolic byproducts from virtually all living cells in the body, the microcirculation plays a critical but indirect role in tissue function. Herein lies a major challenge. The microcirculation must maximize the distribution of nutrients and oxygen by touching nearly every living cell, while minimizing the space it occupies to allow room for the network of parenchymal cells, structural tissue, nerves, inflammatory, and other cell types that contribute more directly to organ function. The circulation comprises only about 7% of the body's volume but is arranged to be ubiquitously distributed. This is achieved by a complex but highly organized branching pattern that can be described in terms of fractal geometry (the pattern has a fractional dimension as a result of the reiterative branching pattern)¹. With diseases such as diabetes, the fractal dimension decreases, possibly contributing to less efficient tissue perfusion^{2, 3}.

Traditionally, studies of the coronary microcirculation have focused on vasoregulation and perfusion in animal models, providing excellent insight into mechanisms of metabolic dilation, biomechanical properties of myocardial perfusion, collateral development, and endothelial regulation of coronary resistance in health and disease. Only recently has technology been sufficient to assess microcirculatory function in intact humans. As a result, it is becoming apparent that the microcirculation is importantly involved in a variety of pathological conditions and that microvascular dysfunction may herald, either as a marker and/or mechanism, the development of cardiovascular disease. This review will discuss the growing recognition that the microcirculation contributes to the development of atherosclerotic cardiovascular disease, review regulation of the coronary microcirculation in humans, and discuss potential novel functions of the microcirculation, beyond regulating perfusion that might explain the intimate link to cardiovascular disease.

Recognition of microvascular disease in humans

One of the difficulties in reading the microvascular literature is that the terms “microvascular disease” and “microvascular dysfunction” refer to a heterogeneous set of conditions ranging from reduced organ maximal perfusion, to impaired endothelium-dependent dilation of isolated arterioles. Microvascular angina is a case in point. This condition signifies “microvascular dysfunction” as a diagnosis of exclusion made in patients presenting with angina pectoris, non-obstructive coronary arteries at catheterization, and no obvious primary etiology⁴. The syndrome was originally thought to represent an abnormality in the coronary microcirculation since it was associated with reduced coronary flow reserve (CFR: peak to resting flow ratio)^{5, 6}, although the definition was later expanded to include patients with normal CFR and a disturbance in pain perception⁷. In this review “microvascular dysfunction” refers to a reduction in maximal vasodilator capacity of the heart.

Earlier studies utilized invasive methods to assess microvascular function (catheterization-based techniques) or expensive, less invasive imaging such as positron emission tomography. Since the development of electron beam computed tomography and cardiac magnetic

resonance imaging, relatively non-invasive, accessible and accurate measurements of coronary perfusion are now possible in humans. This has been instrumental in producing a body of literature identifying reduced CFR as an independent prognostic factor for adverse cardiovascular outcomes⁸⁻¹⁵. Prediction of cardiovascular events may even be greater for microvascular than conduit coronary disease although this finding is not universal¹⁶. Growing recognition that microcirculatory abnormalities contribute to cardiac prognosis supports the need for a more direct and focused understanding of how microvascular function is regulated in humans.

Lanza and colleagues⁴ nicely reviewed the syndrome of primary microvascular angina, highlighting the varied nature of this diagnosis through a classification based on the clinical situation and underlying mechanisms. Microvascular angina can present as a chronic stable condition or as part of an acute coronary syndrome, both associated with microvascular pathology. Microvascular dysfunction may be manifest in the downstream branches of a specific conduit artery, or sporadically across the heart and independent of epicardial artery perfusion territory⁴, making detection difficult. Mild diffuse microvascular dysfunction may not reduce systolic contraction, or evoke classic ischemic ECG changes or scintigraphic perfusion defects, often delaying or obscuring diagnosis. Heightened suspicion and repeated diagnostic evaluation may be needed to establish microvascular dysfunction.

A number of conditions are closely associated with microvascular dysfunction. Diabetes Mellitus manifests multi-organ pathology resulting from microvascular dysfunction. Nahser et al. showed lower CFR and reduced metabolic dilation in diabetes, signs of impaired microvascular function,¹⁷. Di Carli et al. expanded this observation by showing normal baseline flow, but similarly reduced endothelium-dependent and endothelium-independent (dilation to adenosine) microvascular dilator capacity in young subjects with either type I or type II diabetes¹⁸. Reduced dilation to adenosine persisted after controlling for duration of diabetes, insulin treatment and autonomic neuropathy¹⁸.

More direct impairment of microvascular function occurs following sustained ischemia, manifest as reduced maximal flow on CT or MRI in the absence of conduit stenosis. This phenomenon, due to microvascular blockage, is typically seen after stenting with low TIMI flow (angiographic “no reflow”) and is independently associated with major adverse cardiac events¹⁹. The cause of microvascular obstruction is multifactorial including anatomical obstruction (clumped leukocytes or platelets²⁰, perivascular edema and/or cell swelling²¹), reduced vasoreactivity, or frank microvascular necrosis. A combination of intrinsic and extrinsic obstruction may result from intramyocardial hemorrhage which occurs after prolonged ischemia in pigs²². Given that current treatment strategies emphasize early and intense anti-thrombotic treatment following percutaneous interventions, new strategies to minimize downstream microvascular hemorrhage while preventing upstream thrombosis could improve outcomes following myocardial infarction.

It is generally assumed that reperfusion plays a significant role in the magnitude of “no-reflow” and microvascular occlusion. Early and sudden reperfusion (e.g. percutaneous coronary intervention) may reduce the duration of myocardial ischemia but reintroduces blood elements and raises intravascular pressure into a distressed region of the heart with

fragile, often damaged blood vessels. Staged or delayed reperfusion could obviate some of this reperfusion damage, in fact recent provocative data in humans support that idea. In a randomized prospective trial of patients presenting with ST elevation myocardial infarction, a strategy of delayed stenting/reperfusion resulted in less “no-reflow” and greater myocardial salvage²³. Strategies to reduce tissue swelling and avoid leukocyte and/or platelet activation could further minimize post-ischemic microvascular and consequent cardiac damage.

Other conditions associated with microvascular dysfunction are listed in the Table. Given the wide array of diseases for which microvascular dysfunction may play a causal role, identification of biomarkers for microvascular dysfunction will be an important future clinical direction which may unveil novel risk factors for cardiovascular events and improve diagnostic and prognostic assessment.

The microcirculation as a window into conduit artery disease and cardiovascular events

Abnormal microvascular function typically precedes and predicts the development of conduit artery atherosclerosis and its risk factors. Even with minimal coronary artery disease (CAD) acetylcholine-induced vasodilation is impaired^{24, 25}. Framingham risk score is an independent predictor of microvascular dysfunction in patients without obstructive coronary disease²⁶. This is consistent with the idea that the microcirculation is a proverbial “canary in the mine shaft”, affected by risk factors for cardiovascular disease, evident prior to the onset of angiographic atherosclerosis²⁶. Indeed in patients with exertional angina, no obstructive coronary disease and with normal conduit artery endothelial function, there is evidence for microvascular endothelial dysfunction²⁷.

Women, who show a higher prevalence of angina syndromes with normal conduit arteries, have a higher degree of microvascular disease as observed in the Women Ischemia Syndrome Evaluation (WISE) trial of women undergoing evaluation for chest pain. In those with normal conduit coronary arteries, reduced CFR is present but is not associated with traditional risk factors for atherosclerosis^{28, 29}. In these subjects a reduced CFR³⁰ or a reduction in coronary endothelial function²⁹ was independently predictive of future coronary events. Halcox et al. confirmed microvascular endothelial dysfunction as a risk factor for major cardiovascular events independent of coronary atherosclerosis⁹. We speculate that reduced microvascular dilation may predispose tissue to inflammation, facilitating the development of epicardial atherosclerosis. Impaired microvascular dilator capacity may also corrupt the ability to match tissue oxygen demand and supply. This would evoke ischemia and eventually inflammation and vascular proliferation as well as parenchymal cell dysfunction. Particularly susceptible is the heart, where oxygen supply must be linked to demand through rapidly responsive changes in flow. Impaired flow regulation could initiate a viscous inflammatory cycle culminating in myocyte damage and heart failure. Better understanding the mechanism of microvascular dysfunction should help manage conduit artery disease by reversing the pro-atherosclerotic microvascular milieu that may fuel the development of CAD and heart failure.

Microcirculatory dysfunction has been implicated in a wide variety of pathologies (Table). Microvascular dysfunction contributes to inflammation in visceral fat, which may explain the associated elevation in cardiovascular risk in subjects with excess visceral adiposity³¹. Microcirculatory dysfunction contributes etiologically or has a primary association with a myriad of diseases including obstructive sleep apnea³², hypertrophic cardiomyopathy¹⁰, stress-related cardiomyopathy³³, congestive heart failure with reduced ejection fraction³⁴, idiopathic cardiomyopathy³⁵, heart failure with preserved ejection fraction^{36, 37}, inflammatory bowel disease^{38, 39}, schizophrenia⁴⁰, amyloidosis⁴¹, tobacco use⁴², aging⁴³, systemic lupus erythematosus⁴⁴, and even a sedentary life style⁴⁵. Abnormalities in the microcirculation are responsible for no-reflow phenomenon (reviewed by Feher et al.⁴⁶), damage from cardioplegic arrest⁴⁷, coronary microvascular spasm⁴⁸, cerebral vasospasm following subarachnoid hemorrhage⁴⁹, and angiogenesis^{50, 51}, including tumor angiogenesis⁵². To the extent that these associations are confirmed as causal, the implications for treatment and prevention will be substantial. Much of the benefit of statins and angiotensin converting enzyme inhibitors may relate to previously under-considered targets in the microcirculation^{53, 54}. Future clinical trials to evaluate therapies designed to improve microvascular function, may also provide benefit for conduit artery disease and for cardiomyopathies associated with arteriolar dysfunction. To this end, soluble epoxide hydrolase inhibitors, being considered for the treatment of hypertension, increase epoxyeicosatrienoic acids (EET) in human coronary arterioles and can augment vasodilation via these vasoprotective compounds⁵⁵. Potential benefits of enhancing EET release include reduced apoptosis⁵⁶ and inhibition vascular inflammation, thrombosis, and proliferation⁵⁷.

The broad array of diseases associated with microvascular dysfunction has spawned interest in identifying genetic associations. In over 600 patients with non-obstructive conduit coronary arteries, Yoshino et al. sought SNPs from 76 candidate genes. In those with low CFR (<2.5), single nucleotide polymorphisms (SNPs) within VEGFA and CDKN2B (important in vascular cell proliferation) were more prevalent. SNPs for MYH15 (codes for myosin heavy chain), VEGFA, and NT5E (associated with vascular calcification) were associated with low CFR in men but not women. In a prospective study, Fedele et al.⁵⁸ identified specific polymorphisms in the gene for eNOS, and for ion channels including Kir6.2 (K_{ATP} channels), and Nav1.5 (sodium channel) in patients undergoing cardiac catheterization. SNPs in eNOS and in Kir6.2 were specific for subjects with microcirculatory dysfunction. In vasospastic angina or microvascular angina, Mashiba et al.⁵⁹ found a higher frequency of SNPs in paraoxonase 1 (PON1; A632G), but not in other oxidative stress enzymes tested. Slow coronary flow is associated with a polymorphism of eNOS. Stratifying patients by presence or absence of the eNOS4a/b variant predicts low TIMI frame count on angiography in multivariate analysis with an odds ratio of 3.22 ($p<0.05$)⁶⁰. These association studies provide impetus for directed evaluation of these pathways as mechanistically important therapeutic targets in personalized medicine.

The microcirculation can be most easily examined in the periphery including skin, retina, mucosal tissues, and limb vasculature, although these approaches are not often used clinically. Katoh and colleagues showed that the femoral blood flow increases to acetylcholine, indicative of resistance arteriolar endothelial function, correlated with risk factors for cardiovascular disease⁶¹. Endothelial dysfunction in the retinal microcirculation

is also an indicator of risk for atherosclerotic cardiovascular disease⁶². Even changes in the sublingual microvascular anatomy are predictive of complications in patients with acute coronary syndrome⁸. It is becoming evident that changes in microvascular function precede and predict conduit artery pathology. Since assessing coronary microvascular function is technically more challenging than for peripheral arterioles (see below), use of the peripheral microvasculature as a window into systemic and coronary arteriolar function would provide a paradigm shift in how we assess cardiac risk. Future studies should address whether cutaneous, retinal, or mucosal arteriolar function correlates with coronary microvascular function in the same way that brachial artery FMD serves as a viable index of coronary conduit artery endothelial function⁶³.

Assessing human microcirculatory function

Most methods to interrogate human coronary microvascular reactivity are invasive and indirect, measuring target organ flow reserve. In the heart this is typically calculated as CFR, the ratio between maximal flow during reperfusion or pharmacological dilation, and basal flow. CFR can be assessed using intracoronary Doppler, coronary sinus thermodilution (technically challenging and less accurate) or by measuring transit time of radiopaque dyes traversing conduit coronary arteries. This latter method, codified in the Thrombolysis in Myocardial Infarction (TIMI) clinical trials as TIMI flow grade (wash-in speed of dye past a stenosis) or as the more quantifiable corrected TIMI frame count (CTFC) (number of angiographic frames it takes for the leading edge of intracoronary dye to traverse a coronary artery) is relatively easy to measure, correlates with outcomes following reperfusion, and remains widely used today^{64–66}.

Each of these methods can quantitatively detect myocardial no-reflow or microvascular dysfunction. However, these approaches are associated with three major limitations. First, with rare exception direct visualization is not possible using non-invasive techniques. Second, information from these techniques does not differentiate dysfunction within the endothelium from that arising in the VSMC or due to extravascular compression. Third, these techniques are confounded by local neurohumoral influences, circulating substances, and paracrine or mechanical impact from underlying parenchymal cells. Moreover, most techniques require careful training and are costly, making it difficult to include measurements of microcirculatory function in large populations studies⁶⁷. More recently three non-invasive methods have been validated to assess microvascular flow. These include magnetic resonance angiography, (MRA), rapid acquisition CT, and echo contrast displacement. The latter technique involves use of high energy ultrasound to destroy injected microbubbles in the myocardium and assess microvascular flow by observing the time for reopacification of the heart⁶⁸. As a result of these innovations, *in vivo* study of the human microcirculation is now easier but confounding influences of neurohumoral compounds, metabolic dilation, and blood elements persist.

To circumvent many of these problems, vasomotor function can be studied directly *in vitro* using human tissue samples. This review will focus on what has been learned from direct measures of microvascular function in human tissue, including data from animal studies for comparison. This approach provides unprecedented insight into microvascular function in

humans, highlighting mechanisms of disease and opportunities for treating the array of conditions where disturbances in microcirculatory function are thought to play a role (Table).

Direct videomicroscopy using cannulated, pressurized arterioles is a specialized approach for assessing microvascular function in vitro. Fresh tissue is obtained from living subjects either via biopsy or during already planned surgical procedures. Arterioles are dissected from the tissue and cannulated with micropipettes filled with physiological fluid and connected to a reservoir column to maintain an estimated physiological intraluminal pressure. The entire system is monitored with a videomicroscope and intra- and extraluminal fluids can be manipulated independently, allowing changes in vascular diameter to graded chemical or physical stimuli to be quantified directly. Because surgical acquisition of tissue is required, only a few laboratories utilize this technique in studies of human vessels. Over two decades of investigation show that human arteriolar responses are often different from those in animals and establish the importance of using human tissue for determining which animal models best recapitulate the human condition.

Role of the microcirculation in tissue homeostasis

The traditional role of the arterial microcirculation is to regulate vascular resistance and match metabolic demand with blood flow. In the heart this regulation occurs on a second-to-second basis to optimize cardiac performance and prevent ischemia. In addition to the dialog between cardiac metabolism and arteriolar tone, vascular resistance is modulated by a prominent neurohumoral influence during exercise or stress, and by myogenic tone⁶⁹. Myogenic constriction protects the down-stream vasculature from damaging effects of acute elevations in pressure, prevents excessive flow to the perfused tissue and establishes capacity for flow reserve. Other modulators of vascular tone include cell-cell electrical coupling and endothelial derived factors (e.g. nitric oxide [NO], prostacyclin, endothelium-derived hyperpolarization via factors [EDHFs], endothelin-1, thromboxane A₂) that migrate to the underlying smooth muscle and elicit relaxation or contraction. Tremendous species and organ level variation exists with respect to the specific mediator and the relative impact on arteriolar tone. Even along the length of the same coronary vessel, receptor density and responses to vasomotor stimuli can vary^{70, 71}. Conversely in response to the same stimulus, the mediator of dilation can vary across vascular beds or species. For example, arteriolar flow-mediated dilation (FMD) in the porcine coronary arteriolar bed is mediated by NO⁷². In rat cremaster vessels, FMD is mediated by vasodilator prostaglandins⁷³, while in female eNOS null mice, skeletal muscle FMD is due to endothelial release of EETs⁷⁴. In human, dog, and rodent ventricular arterioles, acetylcholine initiates an endothelium-dependent dilation mediated by NO. However in the porcine coronary circulation, an endothelium-independent constriction is seen⁷⁵.

It is fascinating that the body utilizes such a rich cornucopia of endothelial-derived dilator substances to modulate microcirculatory tone, but the teleological importance is not clear. Further insight might be gained from recent evidence for a less traditional role of the microcirculation. Each of the endothelial mediators of vasodilation is released abluminally from arterioles where they act on vascular smooth muscle cells (VSMC) to elicit dilation,

hypertrophy, or fibrosis. These mediators also penetrate to the underlying parenchymal cells especially from the capillary bed with its large surface area and lack of insulation from intervening VSMCs. Thus the vascular organ is ideally situated to exert paracrine effects on the underlying parenchymal tissue.

The microcirculation is architecturally designed for this novel form of local regulation since its intimate support of every organ necessitates close proximity to parenchymal cells. Vascular paracrine regulation of tissue function could help explain the diversity of factors produced by the endothelium (NO, prostacyclin, EETs, hydrogen peroxide [H_2O_2]) which elicit similar degrees of dilation but allow for a variety of local tissue responses including proliferation, fibrosis, apoptosis, and thrombosis. In this new paradigm, the microcirculation not only responds to metabolic cues from the tissue to regulate flow, but conversely the local tissue responds to factors released from the microvessels in response to mechanical and chemical stimuli. For example, nitric oxide (NO) released from endothelial cells during flow diffuses to the underlying parenchymal tissue where it exerts potent inhibition of mitochondrial metabolism, reduces production of reactive oxygen species (ROS), and inhibits inflammation^{76, 77}. The decrease in metabolism in turn reduces cardiac production of metabolic factors that promote dilation. This paracrine influence is bidirectional in that lumenally released NO reduces platelet activation and adhesion molecule expression, thereby inhibiting thrombosis and vascular inflammation^{78, 79}.

Other examples of local paracrine regulation by the endothelium exist. Inhibition of eNOS impairs diastolic cardiac function and promotes hypertrophy⁸⁰, while in pathological situations uncoupled eNOS directly contributes to oxidative stress and associated cardiac dysfunction. During disease or stress, endothelial release of NO is reduced and often replaced by release of endothelin-1, thromboxane A_2 , and ROS which evoke tissue inflammation, inhibit or activate apoptosis, and modulate contractility in cardiomyocytes⁸¹.

When NO bioavailability is reduced, endothelium-dependent dilation is often maintained by compensatory generation of EETs or H_2O_2 ^{74, 82, 83}. EETs are a critical intracellular component of the pathway mediating FMD in patients with CAD⁸⁴, and serve as an EDHF for bradykinin-mediated dilation⁸³. EETs inhibit vascular inflammation and apoptosis in human coronary and pulmonary endothelium⁵⁶, paralleling the non-vasomotor effects of NO. In contrast H_2O_2 , which can also compensate for loss of NO, is pro-inflammatory^{85–87}. If released chronically from endothelial cells, H_2O_2 stimulates smooth muscle cell proliferation, activates endothelial cells, and promotes thrombosis. H_2O_2 can also modulate activity of NO and superoxide⁸⁸. Thus the nature of the compensatory mediator of dilation in disease or in response to stress may not only be important for regulating vascular tone, but may also impact the health of the underlying organ (Figure 1). This effect is primarily a function of the microvasculature since conduit arteries contribute only a small fraction of an organ's endothelial cell volume.

The concept that microvessels contribute to tissue inflammation gains traction from recent studies in adipose tissue. As reviewed by Scalia, vascular dysfunction precedes and mediates tissue inflammation in obesity³¹. Free fatty acids (FFA) are liberated from circulating lipid particles by lipoprotein lipase on the endothelial cell surface after a high fat meal. Since

FFA are poorly taken up by endothelial cells, they initiate acute vascular inflammation in the adipose microcirculation^{31,89}. The resulting endothelial activation promotes adipocyte inflammation, a process aggravated by obesity where the enlarged adipocyte outstrips its blood supply, fueling the hypoxic, inflammatory environment⁹⁰.

Paulus and Tschope have recently proposed that microvascular dysfunction might underlie heart failure with preserved ejection fraction (HFpEF)³⁷. Their intriguing hypothesis posits that coronary microvascular endothelial inflammation from chronic disease (e.g. diabetes, COPD, obesity, hypertension) evokes inflammation via production of excess ROS with reduced NO bioavailability, cGMP and PKG signaling in the underlying myocardium. This concept is supported by the finding of enhanced oxidative stress in endothelial cells and cardiac myocytes in biopsies of patients with HFpEF patients⁹¹. The vascular inflammation induces hyperphosphorylation of titin, promoting cardiomyocyte hypertrophy, stiffness, and fibrosis. Interestingly, the cardiotropic parvovirus B19 which selectively infects endothelial cells, is directly linked to endothelial dysfunction and HFpEF⁹². This new paradigm places the microcirculation at the helm of cardiac disease development, and suggests that we refocus diagnostic, preventive, and therapeutic efforts toward understanding the microcirculatory abnormalities in subjects with heart failure with preserved ejection fraction (HFpEF), a heterogeneous group of patients who have not been responsive to conventional cardiomyopathy- targeted therapies, and for whom the need exists to more fully clarify diagnostic criteria and pathophysiology.

Beyond the autocrine and paracrine functions described, vascular cells, including the endothelium have an ingenious method for communicating with remote downstream vascular elements. Endothelial cells are capable of shedding microvesicles, small membrane-bound fragments (100nm to 1µm in diameter) that contain lipids, proteins, and microRNA⁹³. Microvesicles provide a molecular fingerprint in terms of cell surface proteins and cytosolic contents unique to the parent cell. Once released into the vasculature, these particles bind and interact with other cell types, including downstream endothelial cells in the same tissue segment⁹⁴. These couriers of biological information can then release their contents or activate surface receptors downstream. Circulating microvesicles from patients with cardiovascular disease impair endothelial function in vessels from healthy animals⁹⁵. Although the majority of published studies focus on the pathological actions of microvesicles, recent reports identified particles containing micro RNAs that exert protective effects on downstream targets⁹⁶. Microvesicles appear to be more than just biomarkers of vascular injury. They serve as important para/endocrine mediators in cell-to-cell communication. Given its large volume, the microcirculation probably serves as the major source of endothelial microvesicles in the circulation.

These examples define the microvasculature, the largest paracrine factory within the body, not only as a regulator of blood flow but also as a key modulator of parenchymal cell function in health and disease. It is not surprising that microcirculatory function, especially endothelial, is exquisitely important for maintaining tissue health. Using the stimulus of shear stress, ubiquitous to all endothelial cells in the body, we have begun to uncover conditions where the endothelial paracrine response changes from one that promotes tissue

homeostasis (release of NO) to one that contributes to tissue inflammation and pathology (release of H₂O₂).

Plasticity in Microvascular Signaling

Flow mediated vasodilation refers to a process whereby blood flow activates a mechanochemical signal transduction pathway leading to smooth muscle relaxation and dilation. Present in virtually all vessels, FMD is arguably the most important physiological mechanism of endothelium-dependent vasodilation, serving to facilitate metabolic vasodilation by selectively dilating upstream vessels supplying the site of metabolic activity. A variety of endothelial derived chemical substances or direct electrical conduction to the smooth muscle can mediate FMD, depending on the tissue and species. In most animal models and in normal human tissue, NO is the principal mediator of microvascular FMD. Basal vascular diameter in the human heart is maintained by NO released from the endothelium during shear stress. Interestingly the source of this NO is neuronal nitric oxide synthase (nNOS)⁹⁷, not the traditional endothelial isoform (eNOS). The relatively selective inhibitor of nNOS, S-methyl-L-thiocitrulline, reduces coronary flow but has no effect on dilation to substance P or acetylcholine, both of which were blocked by the non-specific NOS inhibitor (N(G)-monomethyl-L-arginine)⁹⁷⁻⁹⁹, thus both forms of NOS play a role in human endothelial physiology. Similar plasticity occurs in the mouse coronary circulation. Whereas wild type mice utilize eNOS for coronary dilation to flow or acetylcholine, eNOS KO mice recruit nNOS for endothelium-dependent generation of NO^{100, 101}.

Conduit arteries including brachial and coronary vessels also demonstrate FMD. The brachial artery is an excellent index of endothelial NO production since NO is the sole mediator of FMD in that vessel. This is not the case with the downstream radial artery which in addition to NO utilizes products of cytochrome P450 2C9 epoxygenase to elicit FMD¹⁰². The magnitude of brachial FMD is inversely related to the presence of coronary disease, its risk factors, and incidence of future cardiac events¹⁰³⁻¹⁰⁶. Even brief insults such as transient hyperglycemia or elevations in pressure can abrogate brachial artery FMD by reducing bioavailability of NO^{107, 108}. Brachial artery flow mediated dilation is an excellent surrogate for coronary endothelial function⁶³ and has been used extensively in evaluating cardiovascular risk. Endothelium-dependent dilation of adipose arterioles also correlates with brachial FMD providing a link between macro and microvascular dysfunction¹⁰⁹. Future investigation should establish whether adipose arteriolar function can serve as a surrogate for the coronary microcirculation, providing a minimally invasive window into coronary microvascular dysfunction.

In the human microcirculation, endothelium-dependent dilation is reduced in patients with CAD¹¹⁰ but rarely eliminated. Even in subjects with extensive CAD¹¹¹, or in explanted hearts from patients with end-stage heart failure¹¹², basal or -stimulated NO production can be observed. Brief incubation with ACE-inhibitors, statins, or sepiapterin in arterioles from subjects with significant CAD can improve endothelial dependent agonist-induced vasodilation likely by restoration of NO production^{113, 114}. These mechanistic changes in human coronary vascular responsiveness supply critical insight regarding the best animal models of human disease. Such “reverse translation” maximizes relevance of physiological

findings in animal models. To this end, Tiefenbacher et al.¹¹⁴ have shown that a variety of endothelium-dependent agonists stimulate uncoupled eNOS to reduce dilation in both human and pig coronary arteries. In both models an improvement in endothelial function was observed with tetrahydrobiopterin. Shimokawa's laboratory¹¹⁵ has demonstrated H₂O₂ from uncoupled eNOS as an EDHF in the mesenteric circulation of both humans and mice.

We have examined the effect of CAD on coronary arteriolar FMD. In healthy patients, even into their seventh decade, microvascular FMD is mediated by NO¹¹⁶. With the onset of CAD shear stress still elicits dilation, but the mechanism changes¹¹⁷. Instead of NO, shear stimulates production of superoxide leading to H₂O₂ from proximal mitochondrial respiratory complexes¹¹⁸. The mitochondrial derived H₂O₂ is released from the endothelial cell and taken up by the underlying VSMCs where it activates PKG_{1α} by dimerization through oxidation of cysteine 42¹¹⁹. PKG_{1α} opens large conductance calcium-activated potassium (BK_{Ca}) channels to hyperpolarize and relax the smooth muscle. The pathway of endothelial signal transduction is not fully defined but involves activation of endothelial TRPV4¹²⁰ cation channels and requires an intact endothelial cytoskeleton¹²¹. EETs are a necessary component of this pathway likely via intracellular signaling⁸⁴. Thus in CAD, FMD is elicited by H₂O₂. This contrasts with normal subjects where FMD is mediated by NO. The mechanism of this switch in dilator is important as it could impact both coronary flow regulation and tissue homeostasis. Restoration of NO as the mediator of FMD would be expected to maintain dilator capacity but also reduce inflammation, vascular proliferation, and thrombotic potential. We have recently identified two novel and critical components of the microvascular transition from health to disease that could expand therapeutic options for treating or preventing complications of CAD.

Mechanism by which the mediator of FMD changes from NO to H₂O₂ in the presence of CAD

Two pathways involving production of the sphingolipid ceramide and a reduction in telomerase activity are critical in the transition from NO to H₂O₂ as the mediator of FMD in patients with CAD³⁷. Ceramide, produced by neutral sphingomyelinase is a risk factor for atherosclerosis^{122–124}, in part by stimulating ROS generation from mitochondria^{125, 126}. Sphingomyelinase is shear-sensitive¹²⁷ which strategically positions it for a role in shear-induced mitochondrial H₂O₂ production and therefore FMD in the diseased heart.

Treatment of vessels with ceramide overnight is sufficient to invoke a switch in the mechanism of FMD in the human heart from NO to H₂O₂, and reductions in ceramide levels can restore NO as the CAD¹²⁸. These functional changes in heart microvessels were also confirmed in adipose tissue, providing generalizability across organ systems.

One of the downstream effects of ceramide is transcriptional suppression of the catalytic subunit of telomerase (TERT)¹²⁹. Telomerase, typically viewed as a defender of nuclear telomere length, has been identified in the cytosol and mitochondria where it can modulate ROS production^{130, 131}. Outside the nucleus, TERT stimulates NO production from NOS¹³². Mice deficient in the gene for TERT have elevated levels of mitochondrial H₂O₂^{133, 134}. Thus telomerase is capable of influencing production of NO and mitochondrial ROS. Upregulation of telomerase with AGS-499 restores NO as the mediator of FMD in patients

with CAD^{135, 136}. In preliminary studies PPAR γ , an inducer of telomerase, evokes the same restoration in mechanism of dilation¹³⁷. Conversely inhibition of telomerase activity induces the CAD phenotype in normal human arterioles. Thus both ceramide and telomerase regulate the mediator of FMD in the human microcirculation (Figure 2). Understanding pathways of dilation and the associated mediators helps to frame options for maximizing perfusion and minimizing pathological paracrine influences in disease.

Unique properties of the human microcirculation

The human coronary microcirculation exhibits several unique characteristics that have been identified through in vitro studies.

Acetylcholine mediated responses

Many vessels express muscarinic receptors on both the endothelium and smooth muscle, which when activated produce NO-mediated dilation (endothelial receptors) and/or constriction (smooth muscle receptors). Interestingly in cannulated microvessels, even if acetylcholine is administered extraluminally where it contacts the endothelium only after traversing the vascular smooth muscle, the result is dilation¹³⁸. This is observed in vivo as well, where in animals parasympathetic activation elicits a coronary arteriolar vasodilation¹³⁹ that is abrogated by NOS inhibition.

The coronary arteriolar response to acetylcholine is species-dependent. In the dog acetylcholine elicits vasodilation¹³⁸. However the same investigators find that in the pig, only vasoconstriction is seen⁷⁵. The human condition is unique in this regard. In ventricular arterioles, Ach induces an endothelium-dependent vasodilation, but in the atria, only vasoconstriction is seen^{114, 140, 141}. Ach is the only agonist exhibiting this differential effect in atria vs. ventricles, although human atrial vessels are capable of endothelium-dependent dilation to substance P, adenosine diphosphate, shear and bradykinin. Even a NOS-inhibitable endothelium-dependent dilation is observed in human atrial vessels to the peptide hormone adrenomedullin¹⁴², suggesting that the pathway for dilation via NOS is active. This dichotomous response to acetylcholine is observed within the same heart independent of risk factors, and occurs only in humans. Mice, rats, dogs, rabbits, cats, ferrets, and cynomolgus primates have a matched response in vessels from both atria and ventricles (unpublished observations). The pathophysiological impact of atrial constriction to Ach is not clear but may have implications for redistribution of myocardial perfusion during vagal activation or in atrial fibrillation¹⁴³.

Bradykinin-mediated human arteriolar vasodilation

Bradykinin (BK) elicits dilation via NO or EDHF in animal models. However unlike FMD there is a striking influence of gender and tissue site¹⁴⁴. In visceral fat from premenopausal women, dilation to BK is more sensitive than in post-menopausal women or young men of similar age¹⁴⁴. The enhanced dilator capacity is abolished by inhibiting NOS. A similar gender enhancement is observed in arterioles from subcutaneous adipose, but NO does not contribute.

Bradykinin dilation in the coronary circulation of patients with CAD is mediated by an even more unusual mechanism. As shown by Larsen et al., BK stimulates the production of H_2O_2 , an EDHF that opens BK_{Ca} channels in the underlying smooth muscle^{83, 145}. This mechanism is similar to that described for shear stress but NADPH oxidase is the source of superoxide and H_2O_2 , not mitochondria. After blocking NADPH oxidase, a residual dilation to BK is revealed. This compensatory dilation is mediated by EETs, being blocked by addition of CYP450 epoxygenase inhibitors. The EET dilation to BK is only seen in the absence of H_2O_2 , suggesting that H_2O_2 inhibits the epoxygenase. EETs emerge only as a contributing dilator when H_2O_2 levels are low. This was demonstrated directly using human recombinant CYP2C9 and CYP2J2¹⁴⁶.

These observations suggest a novel hierarchical system to explain how the coronary circulation maintains the capacity for endothelium-dependent dilation with progression of disease. Under normal conditions, endothelium-dependent dilation is mediated by NO. We speculate that NO inhibits production of EETs and H_2O_2 under normal conditions. Early in disease with mild elevation in superoxide, NO is quenched and eNOS uncoupled, reducing NO bioavailability. In this setting, EET's are typically produced (release of the NO-mediated inhibition of CYP450) as a compensatory dilator mechanism^{83, 147}. With more severe oxidative stress, H_2O_2 blocks generation of EETs and emerges itself as a tertiary compensatory dilator, being one of the few endothelial derived dilator substances that thrives in an oxidative environment¹⁴⁶. Additional stress may overwhelm these compensatory mechanisms or impair smooth muscle relaxation, leading to reduced dilator capacity.

Adenosine and hypoxia

Adenosine and hypoxia are potent vasodilators that participate in metabolic vasodilation. Animal studies indicate a critical role for ATP-sensitive potassium (K_{ATP}) channels in the dilation to hypoxia and adenosine (reviewed by Quayle et al.¹⁴⁸). In humans the role of K_{ATP} channels is more complex since human coronary arteriolar dilation to adenosine is not blocked by the selective K_{ATP} channel blocker, glibenclamide^{149–151} while dilation to hypoxia is either inhibited¹⁵² or left unchanged¹⁴⁹. Reasons for the difference between studies and across species are not obvious but could relate to chronicity of disease, comorbidities, or differences in the experimental preparations. The variation in response attests to the importance of confirming animal microvascular physiology in human tissue.

Microvascular response to stressors

Transient elevations in blood pressure reduce conduit artery endothelial function¹⁰⁷ and affect the downstream microcirculation¹⁵³. In both sedentary subjects and athletes, dilation of subcutaneous adipose arterioles to flow or acetylcholine is mediated by NO. Following in vitro exposure to 30 minutes of elevated intraluminal pressure FMD is reduced in sedentary subjects but maintained, albeit via H_2O_2 and not NO, in athletes¹⁵³. A similar shift in mediator is seen using acetylcholine as the dilator agonist. It is quite unexpected that in the athlete, acute vascular stress induced by an elevation in intraluminal pressure evokes the same transition (from NO to H_2O_2) in dilator mechanism that occurs in patients with the chronic stress of CAD (see above).

Human gut arterioles show endothelium-dependent dilation but the mechanisms and compensatory factors during stress are unique. As shown by Matoba BK relaxes mesenteric arterioles in humans¹⁵⁴. The principal endothelium-derived mediator is H₂O₂, with a smaller contribution from gap junctions¹⁵⁴. Although the source of H₂O₂ was not defined, the same group found in mouse mesenteric arterioles that H₂O₂, derived from uncoupled NOS is a predominant EDHF¹⁵⁵. Whether this is true in humans remains unknown, but in mucosal arterioles from patients without cardiovascular disease NO and prostacyclin mediate dilation to acetylcholine. Under the stress of inflammatory bowel disease, these vessels respond instead with a frank vasoconstriction to acetylcholine which is offset by an endothelium-independent dilation to acetylcholine via VSMC production of prostaglandin D₂ (PGD₂)¹⁵⁶. The net response is a modest dilation. PGD₂ plays no role in unaffected adjacent regions of bowel¹⁵⁶; rather it is recruited specifically in the setting of inflammatory bowel disease.

Diabetes invokes a potent microvascular stress which can be partially attenuated by strict glycemic control (reviewed in¹⁵⁷). In poorly controlled diabetic subjects, arteriolar dilation is reduced to endothelium-dependent (ADP, substance P) and independent (nitroprusside) agonists compared to non-diabetic and well-controlled subjects¹⁵⁸. It is likely that excess ROS play a role¹⁵⁹. Isolated human coronary arterioles from diabetic subjects with CAD demonstrate impaired dilation to hypoxia and to adenosine when compared to non-diabetic subjects¹⁵². This impairment could contribute to diabetic cardiomyopathy¹⁶⁰. The responses to stress and disease in the human microcirculation highlight the important role of ROS as part of the signaling pathway for endothelium-dependent vasodilation. Additional dilator capacity may be summoned during stress when adrenergic tone is elevated since human coronary arteriolar endothelium expresses functionally significant amounts of beta3-adrenoreceptors¹⁶¹. When activated, they elicit dilation via both NO and smooth muscle hyperpolarization¹⁶¹.

Atrial vs. Ventricular Arteriolar Function

Much of our knowledge regarding human coronary arteriolar function derives from atrial appendage vessels obtained from discarded appendectomy specimens procured during cardiopulmonary bypass procedures. Fresh ventricular tissue is available from explanted hearts, untransplantable donor hearts, or from congenital cardiac surgery. It is therefore important to understand the extent to which atrial arterioles can serve as a surrogate for ventricular arteriolar function. Few studies have directly compared sites, however we have gained experiential data over the past 15 years indicating that with one exception (see section above “acetylcholine-mediated responses”): atrial and ventricular arterioles respond in a directionally similar manner to endothelium-dependent and independent stimuli. Importantly, in ventricular arterioles from normal subjects, NO is the mediator of FMD, but this role diminishes with CAD (unpublished observations). This finding is consistent with observations from Hintze’s laboratory showing that ventricular microvessels from diseased hearts produce less NO to Ach than vessels from non-diseased hearts¹⁶². In the future, it will be important to make better use of explanted and unused donor hearts to directly evaluate ventricular arteriolar responses. An intriguing option for acquiring viable ventricular arterioles is the rapid or “warm” autopsy procedure, used for obtaining cancer tissue within 1–4.5 hours (average 2.8) of in-hospital death¹⁶³.

Role of VSMC in the regulation of human arteriolar tone

EDHFs released by the endothelium elicit dilation by hyperpolarizing underlying VSMCs which in turn blocks calcium entry via L-type Ca^{+2} channels, reducing vascular tone. K^{+} channels regulate membrane potential (E_m) and therefore dilator capacity^{164, 165}. VSMC have a membrane resistance of $>10^8$ Ohms¹⁶⁶, thus even small changes in the electrochemical gradient can significantly alter membrane potential and vascular diameter. Vascular resistance resides mostly in the microcirculation where small changes in arteriolar diameter can result in large changes in conductance and flow as predicted by Poiseuille's law. A 20% reduction in arteriolar diameter yields over 100% increase in resistance and reduces flow by more than 50%.

Four types of K^{+} channels are expressed in arteriolar smooth muscle. (1) Voltage-activated K^{+} channels (K_V) mediate resting E_m and vascular tone, elicit dilation to beta-adrenergic stimulation via cAMP¹⁶⁷, and participate in metabolic dilation¹⁶⁸. (2) Large conductance calcium-activated K^{+} channels (BK_{Ca}) maintain E_m upon changes in intracellular Ca^{+2} levels acting as "brakes" for vasoconstrictor stimuli¹⁶⁹, and are a primary target for EDHF^{55, 170} and activation by calcium sparks in human tissue¹⁷¹. In heightened oxidative states, K_V tend to be down-regulated while BK_{Ca} are upregulated as a compensatory influence¹⁷². (3) K_{ATP} channels respond to changes in cellular metabolism and are expressed both on the sarcolemmal membrane and on the mitochondrial outer membrane. (4) Inwardly rectifying (K_{IR}) channels maintain the electrochemical K^{+} gradient and support metabolic dilation in response to small changes in extracellular potassium¹⁷³. Changes in smooth muscle responsiveness in the human microcirculation are incompletely studied and most information that does exist relates to altered potassium channel responses. Our focus is on K_V and BK_{Ca} since they have been studied more extensively in humans.

K_V channels

K_V channels represent a diverse group of relatively low conductance ion channels several of which ($\text{K}_V1.3$, $\text{K}_V1.4$, $\text{K}_V1.5$, $\text{K}_V3.4$, $\text{K}_V4.2$, $\text{K}_V4.3$, and K_V7) are redox sensitive^{174–178}. Forskolin and isoproterenol open K_V channels by increasing cAMP. This dilation is impaired by high glucose¹⁷⁹ or superoxide¹⁸⁰, and can be restored by scavenging superoxide¹⁸¹. This appears to be a direct effect since single channel opening to forskolin is eliminated by incubation in high glucose^{179, 182}. K_V channel function is also sensitive to hypercholesterolemia^{183, 184}, and to pathophysiologically relevant concentrations of ROS in both animals and humans¹⁸⁵. Elevated levels of ROS in CAD and its associated risk factors decrease expression of K_V channels in animals^{186, 187} possibly by down-regulating the transcription factor Sp1¹⁸⁸.

The effect of disease on K_V channel function in humans is less well studied. K_V channels are redox sensitive, undergoing S-glutathionylation from physiological levels of H_2O_2 ¹⁸⁹ with resulting dilation, but at higher cellular oxidative states, inhibition of dilation is seen¹⁹⁰. These redox changes are particularly prominent in $\text{K}_V1.5$ channels^{191, 192} and their modulation also occurs in the human vasculature^{193–195} where reversible sulfenic modification of cysteine residues are observed¹⁹². Oxidative conditions such as atrial fibrillation are associated with reduced $\text{K}_V1.5$ expression^{194, 195}. The same K_V channel

subtype has recently been implicated by Ohanyan et al.¹⁹⁶ in metabolic dilation, where it couples increases in oxygen consumption with myocardial perfusion in mice.

BK_{Ca} channels

BK_{Ca} channels are activated by both Ca²⁺ and membrane depolarization^{197, 198}. These channels do not contribute to resting myogenic tone^{199, 200}, however, they are activated by a number of endothelium-derived relaxing factors, such as NO²⁰¹ and arachidonic acid metabolites^{202–204}. Although not yet confirmed in humans, BK_{Ca} channels can be activated by calcium sparks generated from mitochondrial release of H₂O₂ near the sarcolemmal membrane²⁰⁵. These channels have been extensively studied in animals where disease tends to upregulate channel expression and function, possibly as a regulatory brake on enhanced calcium entry. For example in hypertension an increase in calcium entry through voltage-dependent calcium channels²⁰⁶ raises myogenic tone which is countered by upregulation in expression of the α -subunit (pore forming) of BK_{Ca} channels in the aorta²⁰⁷ and cerebral microvasculature²⁰⁸ of spontaneously hypertensive rats (SHR). This limits calcium entry by hyperpolarizing the smooth muscle cell membrane, closing L-type calcium channels and attenuating vasoconstriction.

In humans, BK_{Ca} smooth muscle activity is increased with disease. Wiecha et al. showed significantly higher activity of BK_{Ca} channels in human smooth muscle cells obtained from coronary atherosclerotic plaques compared to adjacent uninvolved arterial segments²⁰⁹. This is consistent with a compensatory role for BK_{Ca} in some disease states (atherosclerosis and hypertension). In other pathological conditions, BK_{Ca} channel activity is reduced. Exposure of vessels to high glucose decreases BK_{Ca} activity due to H₂O₂²¹⁰. This effect is mediated by oxidation of a key cysteine residue in the bowl region of the channel²¹¹. Other oxidants, such as peroxynitrite, impair activity of BK_{Ca} channels in VSMC of human coronary arterioles²¹². However the situation is more complex in that the same oxidants (H₂O₂) can also enhance activity of BK_{Ca} channels in human VSMC as described above. It is unclear the circumstances that dictate which action of H₂O₂ is observed. This speaks to the importance of H₂O₂ as a versatile, highly regulated signaling molecule in the circulation.

BK_{Ca} channels are an important final target of the pathway mediating endothelium-dependent dilation in the microcirculation. Several EDHFs including EETs and H₂O₂ activate BK_{Ca} in the underlying smooth muscle²¹³. NO mediated vasodilation involves phosphorylation of PKG_{1 α} which opens BK_{Ca} channels²¹⁴. Disease can modify BK_{Ca}-related vasomotion either directly by modifying expression or sensitivity of K channels, or indirectly by impeding the action of endothelial dilator substances that operate through activation of K-channels. We speculate that the intracellular site of H₂O₂ formation, as well as the amount produced and local antioxidant levels, determine the net effect on channel activity and vascular tone.

BK_{Ca} channels and Kv channels work together to mediate dilation to H₂O₂. Preliminary data from the Zhang lab show that in patients without CAD, H₂O₂ dilation depends upon opening of Kv and BK_{Ca} channels²¹⁵. However in patients with CAD, only BK_{Ca} channels contribute to the response. This could explain the slight attenuation in maximal dilation that occurs in patients with CAD where Kv no longer play a role. It also highlights the

complexities of redox regulation of microvascular function where cardiovascular stress through oxidative mechanisms not only changes the mediator of endothelium-dependent dilation (NO to H₂O₂) but also affects the VSMC potassium channels that respond to H₂O₂. Depending on the amount of ROS produced and the regional intracellular concentration, the net effect can be either impaired dilation or frank constriction.

Conclusion

The human microcirculation which plays a critical role in regulating tissue perfusion, is becoming increasingly recognized as a paracrine modulator of the local tissue environment. This provides a dual role for the multitude of dilator and constrictor factors released from the endothelium that also influence downstream vessels, and parenchymal cell function. Cardiovascular stress and disease expose the dynamic nature of these vascular derived mediators which can be changed either acutely (changes in intraluminal pressure) or chronically (presence of CAD). We have reviewed some of the putative mechanisms by which these changes in dilator pathways occur, providing insight for reversal of the microcirculation-induced pro-atherosclerotic environment associated with chronic disease. A better understanding of these pathways is needed, since corruption of signaling within the microcirculation is now recognized as an etiological factor for a growing number of diseases.

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Nonstandard Abbreviations and Acronyms

ACE	Angiotensin Converting Enzyme
Ach	Acetylcholine
ADP	Adenosine Diphosphate
ATP	Adenosine Triphosphate
BK	Bradykinin
BK_{ca} Channel	Large Conductance Calcium-Activated Potassium Channel
CAD	Coronary Artery Disease
cAMP	Cyclic Adenosine Monophosphate
CDKN2B	Cyclin-Dependent Kinase Inhibitor 2B
CFR	Coronary Flow Reserve
cGMP	Cyclic Guanosine Monophosphate

COPD	Chronic Obstructive Pulmonary Disease
CT	Computerized Tomography
CTFC	Corrected TIMI Frame Count
CVD	Cardiovascular Disease
CYP2C9	Cytochrome P450 2C9
CYP2J2	Cytochrome P450 2J2
CYP450	Cytochrome P450
ECG	Electrocardiogram
EDHF	Endothelial Derived Hyperpolarizing Factor
EET	Epoxyeicosatrienoic Acids
E_m	Membrane Potential
eNOS	Endothelial Nitric Oxide Synthase
FFA	Free Fatty Acid
FMD	Flow Mediated Dilation
H₂O₂	Hydrogen Peroxide
HFpEF	Heart Failure with Preserved Ejection Fraction
HFrfEF	Heart Failure with Reduced Ejection Fraction
K_{ATP} Channel	ATP Sensitive Potassium Channel
Kir	Inwardly-Rectifying Potassium Channel
KO	Knockout
Kv Channel	Voltage Sensitive Potassium Channel
MRA	Magnetic Resonance Angiography
MRI	Magnetic Resonance Imaging
MYH15	Myosin Heavy Chain 15
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
Nav1.5	Cardiac Sodium Channel 1.5
nNOS	Neuronal Nitric Oxide Synthase
NO	Nitric Oxide
NSmase	Neutral Sphingomyelinase
NT5E	Ecto-5'-Nucleotidase
PGD2	Prostaglandin D2
PKG	Protein Kinase G

PON1	Paraoxonase 1
PPARγ	Peroxisome Proliferator-Activated Receptor γ
ROS	Reactive Oxygen Species
RNA	Ribonucleic Acid
SHR	Spontaneously Hypertensive Rat
SNP	Single Nucleotide Polymorphism
TERT	Catalytic Subunit of Telomerase
TIMI	Thrombolysis in Myocardial Infarction Study
TRPV4	Transient Receptor Potential Cation Channel, Subfamily Vanilloid, Member 4
VEGFA	Vascular Endothelial Growth Factor A
VSMC	Vascular Smooth Muscle Cell
WISE	Women Ischemia Syndrome Evaluation

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Figure 1.

In a healthy heart (left), arteriolar endothelium produces NO, prostacyclin, and/or epoxyeicosatrienoic acids as well as low levels of hydrogen peroxide, which support a quiescent non-proliferative state. With onset of disease, flow through the microvasculature releases hydrogen peroxide, creating a pro-inflammatory environment throughout the organ, potentially leading to hypertrophy, fibrosis, and atherosclerosis.

Figure 2.

Proposed mechanism for the stress-induced switch in the mediator of flow-induced dilation. In arterioles from healthy subjects, shear activates production of NO to stimulate dilation and vascular homeostasis (left side of diagram). Vascular stress or presence of coronary disease stimulates pathological basal levels of oxidants and initiates a switch in the mediator of flow-induced dilation from NO to hydrogen peroxide. This switch requires ceramide and a reduction in telomerase. Dilation is maintained but at the expense of vascular inflammation and its consequences.

Table**Conditions Linked to Microvascular Dysfunction**

Ischemic Cardiomyopathy
Diabetes Mellitus
Obstructive Sleep Apnea
HFpEF
HFrEF
Aging
Schizophrenia
Dementia
Peripheral Neuropathy
Chagas Disease
Amyloidosis
Chronic Thromboembolic Pulmonary Hypertension
Stress Related Cardiomyopathy
Systemic Lupus Erythematosus
Cerebral Vasospasm
Tumor Angiogenesis
No-Reflow Phenomenon
Inflammatory Bowel Disease
Tobacco Abuse
Hypertrophic Obstructive Cardiomyopathy
Obesity
Systemic Sclerosis
Hypertension
Idiopathic Cardiomyopathy