It has long been an axiom of mine that the little things are infinitely the most important

—Sir Arthur Conan Doyle

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 vasculoparenchymal 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 reactive oxygen species mediates both 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.

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Key Words: microcirculation ▪ muscle, smooth, vascular ▪ nitric oxide ▪ oxidative stress ▪ vasodilation

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, nonobstructive collateral development, and endothelial regulation of coronary resistance in health and disease. Only recently, technology has 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 or as a mechanism, the development of cardiovascular disease (CVD). This review will discuss the growing recognition that the microcirculation contributes to the development of atherosclerotic CVD, 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 CVD.
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 multiorgan pathology resulting from microvascular dysfunction. Nahser et al.17 showed lower CFR and reduced metabolic dilation in diabetes mellitus, signs of impaired microvascular function. Di Carli et al.18 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 diabetes mellitus. Reduced dilation to adenosine persisted after controlling for duration of diabetes mellitus, insulin treatment, and autonomic neuropathy.18

More direct impairment of microvascular function occurs following sustained ischemia, manifest as reduced maximal flow on computerized tomography or magnetic resonance imaging in the absence of conduit stenosis. This phenomenon, due to microvascular blockage, is typically seen after stenting with low thrombolyis in myocardial infarction (TIMI) flow (angiographic no-reflow) and is independently associated with major adverse cardiac events.19 The cause of microvascular obstruction is multifactorial including anatomical obstruction (clumped leukocytes or platelets20 and perivascular edema or cell swelling21), reduced vasoreactivity, or frank microvascular necrosis. A combination of intrinsic and extrinsic obstructions may result from intramyocardial hemorrhage, which occurs after prolonged ischemia in pigs.22 Given that current treatment strategies emphasize early and intense antithrombotic 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 (eg, 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 salvage.23 Strategies to reduce tissue swelling and avoid leukocyte or platelet activation could further minimize posts 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 that may unveil novel risk factors for cardiovascular events and improve diagnostic and prognostic assessment.

### Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>BKca channel</td>
<td>Large conductance calcium-activated potassium channel</td>
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<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
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<tr>
<td>CFR</td>
<td>Coronary flow reserve</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>EDHF</td>
<td>Endothelium-derived hyperpolarization factor</td>
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<tr>
<td>EET</td>
<td>Epoxyeicosatrienoic acids</td>
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<tr>
<td>fmax</td>
<td>Membrane potential</td>
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<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
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<td>FMD</td>
<td>Flow-mediated dilation</td>
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<tr>
<td>HFpEF</td>
<td>Heart failure with preserved ejection fraction</td>
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<tr>
<td>K_channels</td>
<td>Adenosine triphosphate-sensitive potassium channel</td>
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<tr>
<td>Kv_channels</td>
<td>Voltage-sensitive potassium channel</td>
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<tr>
<td>NO</td>
<td>Nitric oxide</td>
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<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
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<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
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<tr>
<td>TIMI</td>
<td>Thrombolysis in myocardial infarction study</td>
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<tr>
<td>VSMC</td>
<td>Vascular smooth muscle cell</td>
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Coronary arteries at catheterization, and no obvious primary etiology.4 The syndrome was originally thought to represent an abnormality in the coronary microcirculation because 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 perception.7 In this review microvascular dysfunction refers to a reduction in maximal vasodilator capacity of the heart.

Earlier studies used invasive methods to assess microvascular function (catheterization-based techniques) or expensive, less invasive imaging such as a positron emission tomography. Since the development of electron beam computed tomography and cardiac magnetic resonance imaging, relatively noninvasive, 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 outcomes.8-15 Prediction of cardiovascular events may even be greater for microvascular than conduit coronary disease, although this finding is not universal.16 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 Crea4 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 territory,4 making detection difficult. Mild diffuse microvascular dysfunction may not reduce systolic
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. Framingham risk score is an independent predictor of microvascular dysfunction in patients without obstructive coronary disease. This is consistent with the idea that the microvascular dysfunction is a proverbial canary in the mine shaft, affected by risk factors for CVD, evident before the onset of angiographic atherosclerosis. Indeed in patients with exertional angina, no obstructive coronary disease, and with normal conduit artery endothelial function, there is evidence for microvascular endothelial dysfunction.

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. In these subjects, a reduced CFR or a reduction in coronary endothelial function 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 atherosclerosis. 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 proatherosclerotic 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 adiposity. Microcirculatory dysfunction contributes etiologically or has a primary association with a myriad of diseases including obstructive sleep apnea, hypertrophic cardiomyopathy, stress-related cardiomyopathy, congestive heart failure with reduced ejection fraction, idiopathic cardiomyopathy, heart failure with preserved ejection fraction (HFpEF), inflammatory bowel disease, schizophrenia, amyloidosis, tobacco use, aging, systemic lupus erythematosus, and even a sedentary life style. Abnormalities in the microcirculation are responsible for no-reflow phenomenon (reviewed by Feher et al), damage from cardiogenic arrest, coronary microvascular spasm, cerebral vasospasm following subarachnoid hemorrhage, and angiogenesis, including tumor angiogenesis.

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 underconsidered targets in the microcirculation. 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 (EETs) in human coronary arterioles and can augment vasodilation via these vasoprotective compounds. Potential benefits of enhancing EET release include reduced apoptosis and inhibition of vascular inflammation, thrombosis, and proliferation.

The broad array of diseases associated with microvascular dysfunction has spawned interest in identifying genetic associations. In >600 patients with nonobstructive conduit coronary arteries, Yoshino et al sought single nucleotide polymorphisms (SNPs) from 76 candidate genes. In those with low CFR (<2.5), SNPs within vascular endothelial growth factor A and cyclin-dependent kinase inhibitor 2B were more prevalent. SNPs for MYH15 (codes for myosin heavy chain), vascular endothelial growth factor (VEGF), and cyclin-dependent kinase inhibitor 2B (important in vascular calcification) were more prevalent. SNPs within vascular endothelial growth factor A and ecto-5′-nucleotidase (associated with vascular calcification) were associated with low CFR in men but not in women. In a prospective study, Fedele et al identified specific polymorphisms in the gene for endothelial nitric oxide synthase (eNOS), and for ion channels including inwardly rectifying potassium channel (Kir6.2; K_ATP channels) and cardiac sodium channel (Nav1.5) 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 found a higher frequency of SNPs in paroxonase 1 (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.

<table>
<thead>
<tr>
<th>Table. Conditions Linked to Microvascular Dysfunction</th>
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<tr>
<td>Ischemic cardiomyopathy</td>
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<tr>
<td>Diabetes mellitus</td>
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<tr>
<td>Obstructive sleep apnea</td>
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<td>HFpEF</td>
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<td>HFrEF</td>
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<tr>
<td>Aging</td>
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<tr>
<td>Schizophrenia</td>
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<td>Dementia</td>
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<td>Peripheral neuropathy</td>
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<td>Chagas disease</td>
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<tr>
<td>Amyloidosis</td>
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<td>Chronic thromboembolic pulmonary hypertension</td>
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HFpEF indicates heart failure with preserved ejection fraction; and HFrEF, heart failure with reduced ejection fraction.
with an odds ratio of 3.22 ($P<0.05$). These association studies provide impetus for directed evaluation of these pathways as mechanistically important therapeutic targets in personalized medicine.

The microrcirculation 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 et al showed that the femoral blood flow increases to acetylcholine, indicative of resistance arteriolar endothelial function, correlated with risk factors for CVD. Endothelial dysfunction in the retinal microrcirculation is also an indicator of risk for atherosclerotic CVD. Even changes in the sublingual microvascular anatomy are predictive of complications in patients with acute coronary syndrome. It is becoming evident that changes in microvascular function precede and predict conduit artery pathology. Because 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 flow-mediated dilation (FMD) serves as a viable index of coronary conduit artery endothelial function.

### 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 TIMI clinical trials as TIMI flow grade (wash-in speed of dye past a stenosis) or as the more quantifiable corrected TIMI frame count (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.

Each of these methods can quantitatively detect myocardial no-reflow or microvascular dysfunction. However, these approaches are associated with 3 major limitations. First, with rare exception, direct visualization is not possible using noninvasive techniques. Second, information from these techniques does not differentiate dysfunction within the endothelium from that arising in the vascular smooth muscle cell (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 microcircular function in large population studies. More recently, 3 noninvasive methods have been validated to assess microvascular flow. These include magnetic resonance angiography, rapid acquisition computerized tomography, 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 heart. As a result of these innovations, in vivo study of the human microrcirculation 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 microprobes filled with physiological fluid and connected to a reservoir column to maintain an estimated intraluminal pressure. The entire system is monitored with a video microscope, and intra- and extravascular 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 use this technique in studies of human vessels. Over 2 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 dialogue between cardiac metabolism and arteriolar tone, vascular resistance is modulated by a prominent neurohumoral influence during exercise or stress and by myogenic tone. Myogenic constriction protects the downstream 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 (eg, nitric oxide [NO], prostacyclin, endothelium-derived hyperpolarization factors [EDHFs], endothelin-1, and 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. Conversely, in response to the same stimulus, the mediator of dilation can vary across vascular beds or species.
For example, arteriolar FMD in the porcine coronary arteriole is mediated by NO. In rat cremaster vessels, FMD is mediated by vasodilator prostaglandins, whereas in female eNOS null mice, skeletal muscle FMD is because of endothelial release of EETs. 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 seen.

It is fascinating that the body uses 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 abuminally from arterioles where they act on VSMCs 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 because 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, and hydrogen peroxide [H2O2]), 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, 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. The decrease in metabolism in turn reduces cardiac production of metabolic factors that promote dilation. This paracrine influence is bidirectional in that luminally released NO reduces platelet activation and adhesion molecule expression, thereby inhibiting thrombosis and vascular inflammation.

Other examples of local paracrine regulation by the endothelium exist. Inhibition of eNOS impairs diastolic cardiac function and promotes hypertrophy; likewise, 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 A2, and ROS, which evoke tissue inflammation, inhibit or activate apoptosis, and modulate contractility in cardiomyocytes. When NO bioavailability is reduced, endothelium-dependent dilation is often maintained by compensatory generation of EETs or H2O2. EETs are a critical intracellular component of the pathway mediating FMD in patients with CAD and serve as an EDHF for bradykinin-mediated dilation. EETs inhibit vascular inflammation and apoptosis in human coronary and pulmonary endothelium, paralleling the nonvasomotor effects of NO. In contrast, H2O2, which can also compensate for loss of NO, is proinflammatory. If released chronically from endothelial cells, H2O2 stimulates smooth muscle cell proliferation, activates endothelial cells, and promotes thrombosis. H2O2 can also modulate activity of NO and superoxide. 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 because 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 obesity. Free fatty acids are liberated from circulating lipid particles by lipoprotein lipase on the endothelial cell surface after a high fat meal. Because free fatty acids are poorly taken up by endothelial cells, they initiate acute vascular inflammation in the adipose microcirculation. The resulting endothelial activation promotes adipocyte inflammation, a process aggravated by obesity where the enlarged adipocyte outstrips its blood supply, fueling the hypoxic, and inflammatory environment.

Paulus and Tschope have recently proposed that microvascular dysfunction might underlie HFrEF. Their intriguing hypothesis posits that coronary microvascular endothelial inflammation from chronic disease (eg, diabetes mellitus, chronic obstructive pulmonary disease, obesity, and hypertension) evokes inflammation via production of excess ROS with reduced NO bioavailability, cyclic guanosine monophosphate, and protein kinase G 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 HFrEF patients. 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 HFrEF. 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 HFrEF, 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 and small membrane-bound fragments (100 nm to 1 μm in diameter) that contain lipids, proteins, and microRNA. 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 segment. These couriers of
biological information can then release their contents or activate surface receptors downstream. Circulating microvesicles from patients with CVD impair endothelial function in vessels from healthy animals. Although the majority of published studies focus on the pathological actions of microvesicles, recent reports identified particles containing microRNAs that exert protective effects on downstream targets. Microvesicles seem to be more than just biomarkers of vascular injury. They serve as important paracrine/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 $H_2O_2$).

**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 NO synthase (NOS), not the traditional endothelial isoform (eNOS). The relatively selective inhibitor of neuronal NOS, $S$-methyl-$L$-thiocitrulline, reduces coronary flow but has no effect on dilation to substance P or acetylcholine, both of which were blocked by the nonspecific 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. Although wild-type mice use eNOS for coronary dilation to flow or acetylcholine, eNOS knockout mice recruit neuronal NOS for endothelium-dependent generation of NO.$^{100,101}$

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**Figure 1.** In a healthy heart (left), arteriolar endothelium produces NO, prostacyclin (PGI$_2$), and epoxyeicosatrienoic acids (EETs) as well as low levels of hydrogen peroxide, which support a quiescent nonproliferative state. With onset of disease, flow through the microvasculature releases hydrogen peroxide, creating a proinflammatory environment throughout the organ, potentially leading to hypertrophy, fibrosis, and atherosclerosis. NO indicates nitric oxide.

**Figure 2.** Proposed mechanism for the stress-induced switch in the mediator of flow-induced dilation. In arteries 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. Ang II indicates angiotensin II; CAD, coronary artery disease, HTN, hypertension; NO, nitric oxide; and TERT, catalytic subunit of telomerase.
Conduit arteries including brachial and coronary vessels also demonstrate FMD. The brachial artery is an excellent index of endothelial NO production because 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 uses products of cytochrome P450 2C9 epoxygenase to elicit FMD. The magnitude of brachial FMD is inversely related to the presence of coronary disease, its risk factors, and incidence of future cardiac events. Even brief insults such as transient hyperglycemia or elevations in pressure can abrogate brachial artery FMD by reducing bioavailability of NO. Brachial artery FMD is an excellent surrogate for coronary endothelial function 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 dysfunction. 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 CAD but rarely eliminated. Even in subjects with extensive CAD, or in explanted hearts from patients with end-stage heart failure, basal or stimulated NO production can be observed. Brief incubation with angiotensin converting enzyme inhibitors, statins, or sepiapterin in arterioles from subjects with significant CAD can improve endothelial dependent agonist-induced vasodilation likely by restoration of NO production. 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 have shown that a variety of endothelium-dependent agonists stimulate uncoupled eNOS to reduce dilation in both human and pig coronary arterioles. In both models, an improvement in endothelial function was observed with tetrahydrobiopterin. Shimokawa’s laboratory 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 NO. With the onset of CAD, shear stress still elicits dilation, but the mechanism changes. Instead of NO, shear stimulates production of superoxide leading to H2O2 from proximal mitochondrial respiratory complexes. The mitochondrial-derived H2O2 is released from the endothelial cell and taken up by the underlying VSMCs where it activates protein kinase G1 (PKG1α) by dimerization through oxidation of cysteine 42. PKG1α opens large conductance calcium-activated potassium (BKCa) channels to hyperpolarize and relax the smooth muscle. The pathway of endothelial signal transduction is not fully defined but involves activation of endothelial transient receptor potential cation channel, subfamily vanilloid, member 4 (TRPV4) cation channels and requires an intact endothelial cytoskeleton. EETs are a necessary component of this pathway likely via intracellular signaling. 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 not only to maintain dilator capacity but also reduce inflammation, vascular proliferation, and thrombotic potential. We have recently identified 2 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 CAD. Ceramide produced by neutral sphingomyelinase is a risk factor for atherosclerosis, in part by stimulating ROS generation from mitochondria. Sphingomyelinase is shear sensitive, 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 mediator of dilation in CAD. 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. Telomerase, typically viewed as a defender of nuclear telomere length, has been identified in the cytosol and mitochondria where it can modulate ROS production. Outside the nucleus, catalytic subunit of telomerase stimulates NO production from NOS. Mice deficient in the gene for catalytic subunit of telomerase have elevated levels of mitochondrial H2O2. 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. In preliminary studies, peroxisome proliferator-activated receptor γ, an inducer of telomerase, evokes the same restoration in the mechanism of dilation. 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 the smooth muscle, which when activated produce NO-mediated dilation (endothelial receptors) 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 dilation.138 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 vasodilation.138 However, the same investigators find that in the pig, only vasoconstriction is seen.75 The human condition is unique in this regard. In ventricular arterioles, acetylcholine induces an endothelium-dependent vasodilation, but in the atria, only vasoconstriction is seen.144,145,146 Acetylcholine is the only agonist exhibiting this differential effect in atria versus 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 adenomodulin,142 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 (D.D. Gutterman, unpublished observations, 1997). The pathophysiological impact of atrial constriction to acetylcholine is not clear but may have implications for redistribution of myocardial perfusion during vagal activation or in atrial fibrillation.143

Bradykinin-Mediated Human Arteriolar Vasodilation

Bradykinin elicits dilation via NO or EDHF in animal models. However, unlike FMD, there is a striking influence of gender and tissue site.148 In visceral fat from premenopausal women, dilation to bradykinin is more sensitive than in postmenopausal women or young men of similar age.144 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,83 bradykinin stimulates the production of H2O2, an EDHF that opens BKcα channels in the underlying smooth muscle.145 This mechanism is similar to that described for shear stress, but nicotinamide adenine dinucleotide phosphate oxidase is the source of superoxide and H2O2, not mitochondria. After blocking nicotinamide adenine dinucleotide phosphate oxidase, a residual dilation to bradykinin is revealed. This compensatory dilation is mediated by EETs, being blocked by addition of cytochrome P450 epoxygenase inhibitors. The EET dilation to bradykinin is only seen in the absence of H2O2, suggesting that H2O2 inhibits the epoxygenase. EETs emerge only as a contributing dilator when H2O2 levels are low. This was demonstrated directly using human recombinant cytochrome P2C9 and cytochrome P2J2.146

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 H2O2 under normal conditions. Early in disease with mild elevation in superoxide, NO is quenched and eNOS uncoupled, reducing NO bioavailability. In this setting, EETs are typically produced (release of the NO-mediated inhibition of cytochrome P450) as a compensatory dilator mechanism.83,147 With more severe oxidative stress, H2O2 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 environment.148 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 adenosine triphosphate-sensitive potassium (KATP) channels in the dilation to hypoxia and adenosine (reviewed by Quayle et al148). In humans, the role of KATP channels is more complex because human coronary arteriolar dilation to adenosine is not blocked by the selective KATP channel blocker, glibenclamide,149–151 whereas dilation to hypoxia is either inhibited152 or left unchanged.149 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 tests 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 microcirculation.155 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 H2O2 and not NO, in athletes.153 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 H2O2) 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 et al,154 bradykinin relaxes mesenteric arterioles in humans. The principal endothelium-derived mediator is H2O2, with a smaller contribution from gap junctions.154 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 EDHF.155 Whether this is true in humans remains unknown, but in mucosal arterioles from patients without CVD, 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 D2.156 The net response is a modest dilation. Prostaglandin D2 plays no role...
in unaffected adjacent regions of bowel, rather it is recruited specifically in the setting of inflammatory bowel disease.

Diabetes mellitus invokes a potent microvascular stress, which can be partially attenuated by strict glycemic control (reviewed by Singh et al157). In poorly controlled diabetic subjects, arteriolar dilation is reduced to endothelium-dependent (adenosine diphosphate, substance P) and independent (nitroprusside) agonists compared with nondiabetic and well-controlled subjects.158 It is likely that excess ROS play a role.159 Isolated human coronary arterioles from diabetic subjects with CAD demonstrate impaired dilation to hypoxia and to adenosine when compared with nondiabetic subjects.152 This impairment could contribute to diabetic cardiomyopathy. 

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 because human coronary arterioles express functionally significant amounts of β-3-adrenoceptors.160 When activated, they elicit dilation via both NO and smooth muscle hyperpolarization.161

**Atrial Versus 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 Acetylcholine-Mediated Responses section in this article): 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.161a This finding is consistent with observations from Hintze’s laboratory showing that vascular resistance resides in unaffected adjacent regions of bowel; rather it is recruited specifically in the setting of inflammatory bowel disease.

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**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 Ca2+ channels, reducing vascular tone. K+ channels regulate membrane potential (E_m) and, therefore, dilator capacity.164,165 VSMCs have a membrane resistance of >10^8 ohms; thus, even small changes in the electrophysical 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 >100% increase in resistance and reduces flow by >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, eliciting dilation to β-adrenergic stimulation via cyclic adenosine monophosphate,167 and participate in metabolic dilation.168 (2) BK Ca channels maintain E_m upon changes in intracellular Ca2+ levels acting as brakes for vasoconstrictor stimuli and are a primary target for EDHFs169,170 and activation by calcium sparks in human tissue.171 In heightened oxidative states, voltage-sensitive potassium (K_v) channel tend to be downregulated, whereas BK Ca are upregulated as a compensatory influence.172 (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 potassium.173 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 because 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 (K_v1.3, K_v1.4, K_v1.5, K_v3.4, K_v4.2, K_v4.3, and K_v7) are redox sensitive.174–178 Forskolin and isoproterenol open K_v channels by increasing cyclic adenosine monophosphate. This dilation is impaired by high glucose179 or superoxide180 and can be restored by scavenging superoxide.181 This seems to be a direct effect because single channel opening to forskolin is eliminated by incubation in high glucose.179,182 K_v channel function is also sensitive to hypercholesterolemia183,184 and to pathophysiology relevant concentrations of ROS in both animals and humans.185 Elevated levels of ROS in CAD and its associated risk factors decrease expression of K_v channels in animals186,187 possibly by downregulating the transcription factor Sp1.188

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 with resulting dilation, but at higher cellular oxidative states, inhibition of dilation is seen.190 These redox changes are particularly prominent in K_v1.5 channels,191,192 and their modulation also occurs in the human vasculature193,194 where reversible sulfenic modification of cysteine residues is observed.192 Oxidative conditions, such as atrial fibrillation, are associated with reduced K_v1.5 expression.194,195 The same K_v channel subtype has recently been implicated by Ohanyan et al196 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; however, they are activated by a number of endothelium-derived relaxing factors, such as NO and arachidonic acid metabolites. Although not yet confirmed in humans, BKCa channels can be activated by calcium sparks generated from mitochondrial release of H2O2 near the sarclemmal membrane. 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 channels raises myogenic tone, which is countered by upregulation in expression of the α-subunit (pore forming) of BKCa channels in the aorta and cerebral microvasculature of spontaneously hypertensive rats. This limits calcium entry by hyperpolarizing the smooth muscle cell membrane, closing L-type calcium channels and attenuating vasoconstriction.

In humans, BKCa smooth muscle activity is increased with disease. Wiecha et al showed significantly higher activity of BKCa channels in human smooth muscle cells obtained from coronary atherosclerotic plaques compared with adjacent uninvolved arterial segments. This is consistent with a compensatory role for BKCa in some disease states (atherosclerosis and hypertension). In other pathological conditions, BKCa channel activity is reduced. Exposure of vessels to high glucose decreases BKCa activity because of H2O2. This effect is mediated by oxidation of a key cysteine residue in the bowl region of the channel. Other oxidants, such as peroxynitrite, impair activity of BKCa channels in VSMC of human coronary arterioles. However, the situation is more complex in that the same oxidants (H2O2) can also enhance activity of BKCa channels in human VSMC as described above. It is unclear 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.

BKCa channels are an important final target of the pathway mediating endothelium-dependent dilation in the microcirculation. Several EDHF-s, including EETs and H2O2, activate BKCa in the underlying smooth muscle. NO-mediated vasodilation involves phosphorylation of protein kinase G, which opens BKCa channels. Disease can modify BKCa-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, determines the net effect on channel activity and vascular tone.

BKCa channels and K channels work together to mediate dilation to H2O2. Preliminary data from the Zhang lab show that in patients without CAD, H2O2 dilation depends on opening of K and BKCa channels. However, in patients with CAD, only BKCa channels contribute to the response. This could explain the slight attenuation in maximal dilation that occurs in patients with CAD where K no longer plays 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.

**Conclusions**

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 proatherosclerotic environment associated with chronic disease. A better understanding of these pathways is needed because corruption of signaling within the microcirculation is now recognized as an etiological factor for a growing number of diseases.

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**Disclosures**

None.

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