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 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. (*Circ Res*. 2016;118:157-172. DOI: 10.1161/CIRCRESAHA.115.305364.)**

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

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 mellitus, 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, 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.

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

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Nonstandard Abbreviations and Acronyms	
BKca channel	large conductance calcium-activated potassium channel
CAD	coronary artery disease
CFR	coronary flow reserve
CVD	cardiovascular disease
EDHF	endothelium-derived hyperpolarization factor
EET	epoxyeicosatrienoic acids
E_m	membrane potential
eNOS	endothelial nitric oxide synthase
FMD	flow-mediated dilation
HFpEF	heart failure with preserved ejection fraction
K_{ATP} channel	adenosine triphosphate-sensitive potassium channel
Kv channel	voltage-sensitive potassium channel
NO	nitric oxide
NOS	nitric oxide synthase
ROS	reactive oxygen species
SNP	single nucleotide polymorphism
TIMI	thrombolysis in myocardial infarction study
VSMC	vascular smooth muscle cell

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 Crea[4] 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 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 thrombolysis 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 platelets[20] and perivascular edema or cell swelling[21]), 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 postischemic 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.

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Table. Conditions Linked to Microvascular Dysfucntion

Ischemic cardiomyopathy	Stress-related cardiomyopathy
Diabetes mellitus	Systemic lupus erythematosis
Obstructive sleep apnea	Cerebral vasospasm
HFpEF	Tumor angiogenesis
HFrEF	No-reflow phenomenon
Aging	Inflammatory bowel disease
Schizophrenia	Tobacco abuse
Dementia	Hypertrophic obstructive cardiomyopathy
Peripheral neuropathy	Obesity
Chagas disease	Systemic sclerosis
Amyloidosis	Hypertension
Chronic thromboembolic pulmonary hypertension	Idiopathic cardiomyopathy

HFpEF indicates heart failure with preserved ejection fraction; and HFrEF, heart failure with reduced ejection fraction.

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 disease.[26] This is consistent with the idea that the microcirculation is a proverbial canary in the mine shaft, affected by risk factors for CVD, evident before the onset of angiographic atherosclerosis.[26] Indeed in patients with exertional angina, no obstructive coronary disease, and with normal conduit artery endothelial function, there is evidence for microvascular endothelial dysfunction.[27]

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 CFR[30] or a reduction in coronary endothelial function[29] was independently predictive of future coronary events. Halcox et al[9] 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.[31] Microcirculatory dysfunction contributes etiologically or has a primary association with a myriad of diseases including obstructive sleep apnea,[32] hypertrophic cardiomyopathy,[10] stress-related cardiomyopathy,[33] congestive heart failure with reduced ejection fraction,[34] idiopathic cardiomyopathy,[35] heart failure with preserved ejection fraction (HFpEF),[36,37] inflammatory bowel disease,[38,39] schizophrenia,[40] amyloidosis,[41] tobacco use,[42] aging,[43] systemic lupus erythematosus,[44] and even a sedentary life style.[45] Abnormalities in the microcirculation are responsible for no-reflow phenomenon (reviewed by Feher et al[46]), damage from cardioplegic arrest,[47] coronary microvascular spasm,[48] cerebral vasospasm following subarachnoid hemorrhage,[49] and angiogenesis,[50,51] including tumor angiogenesis.[52] 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.[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 (EETs) in human coronary arterioles and can augment vasodilation via these vasoprotective compounds.[55] Potential benefits of enhancing EET release include reduced apoptosis[56] and inhibition of vascular inflammation, thrombosis, and proliferation.[57]

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[57a] 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 (important in vascular cell proliferation) were more prevalent. SNPs for MYH15 (codes for myosin heavy chain), 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[58] 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[59] found a higher frequency of SNPs in paraoxonase 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

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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 et al[61] 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 microcirculation is also an indicator of risk for atherosclerotic CVD.[62] Even changes in the sublingual microvascular anatomy are predictive of complications in patients with acute coronary syndrome.[8] 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.[63]

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.[64–66]

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 microcirculatory function in large populations studies.[67] 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.[68] 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 video microscope, 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 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.[69] 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 A_2) 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.

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For example, arteriolar FMD in the porcine coronary arteriolar bed is mediated by NO.[72] In rat cremaster vessels, FMD is mediated by vasodilator prostaglandins,[73] whereas in female eNOS null mice, skeletal muscle FMD is because of endothelial release of EETs.[74] 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 seen.[75]

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 abluminally 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 [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, 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 luminally 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 hypertrophy[80]; 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 A_2, and ROS, which evoke tissue inflammation, inhibit or activate apoptosis, and modulate contractility in cardiomyocytes.[81]

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 CAD[84] and serve as an EDHF for bradykinin-mediated dilation.[83] EETs inhibit vascular inflammation and apoptosis in human coronary and pulmonary endothelium,[56] paralleling the nonvasomotor effects of NO. In contrast, H_2O_2, which can also compensate for loss of NO, is proinflammatory.[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 superoxide.[88] 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,[31] 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.[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, and inflammatory environment.[90]

Paulus and Tschope[37] have recently proposed that microvascular dysfunction might underlie HFpEF. 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 underling myocardium. This concept is supported by the finding of enhanced oxidative stress in endothelial cells and cardiac myocytes in biopsies of patients with HFpEF patients.[91] 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 HFpEF.[92] 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 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 and small membrane-bound fragments (100 nm to 1 μm in diameter) that contain lipids, proteins, and microRNA.[93] 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.[94] These couriers of

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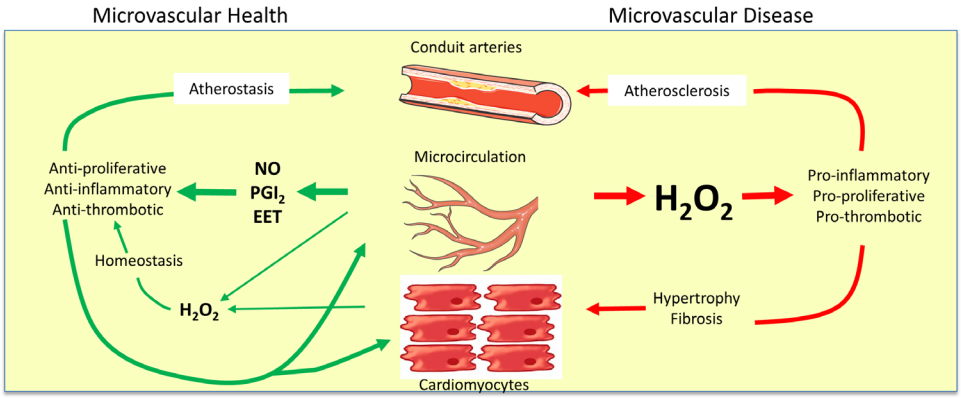

Figure 1. In a healthy heart (left), arteriolar endothelium produces NO, prostacyclin (PGI_2), and epoxyeicosatrienoic acids (EETs) as well as low levels of hydrogen peroxide, which support a quiescent nonproliferative state. With onset of disease, flow through the microvasculature releases hydrogen peroxide, creating a proinflammatory environment throughout the organ, potentially leading to hypertrophy, fibrosis, and atherosclerosis. NO indicates nitric oxide.

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.[95] 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.[96] 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),[97] 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]

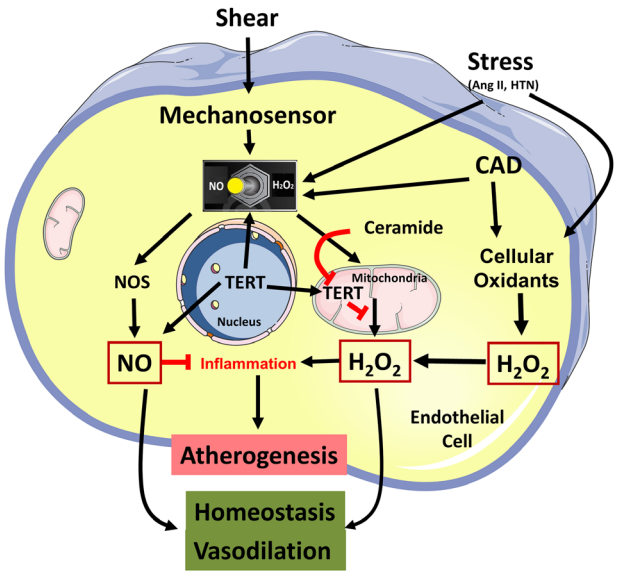

Figure 2. Proposed mechanism for the stress-induced switch in the mediator of flow-induced dilation. In arterioles from healthy subjects, shear activates production of NO to stimulate dilation and vascular homeostasis (left side of diagram). Vascular stress or presence of coronary disease stimulates 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. Ang II indicates angiotensin II; CAD, coronary artery disease, HTN, hypertension; NO, nitric oxide; and TERT, catalytic subunit of telomerase.

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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.[102] The magnitude of brachial FMD is inversely related to the presence of coronary disease, its risk factors, and incidence of future cardiac events.[103–106] 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 FMD is an excellent surrogate for coronary endothelial function[63] 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.[109] 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[110] but rarely eliminated. Even in subjects with extensive CAD,[111] or in explanted hearts from patients with end-stage heart failure,[112] 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.[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 laboratory[115] has demonstrated H_2O_2 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.[116] With the onset of CAD, shear stress still elicits dilation, but the mechanism changes.[117] Instead of NO, shear stimulates production of superoxide leading to H_2O_2 from proximal mitochondrial respiratory complexes.[118] The mitochondrial-derived H_2O_2 is released from the endothelial cell and taken up by the underlying VSMCs where it activates protein kinase $G1_\alpha$ ($PKG1_\alpha$) by dimerization through oxidation of cysteine 42.[119] $PKG1_\alpha$ 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 transient receptor potential cation channel, subfamily vanilloid, member 4[120] cation channels and requires an intact endothelial cytoskeleton.[121] EETs are a necessary component of this pathway likely via intracellular signaling.[84] Thus in CAD, FMD is elicited by H_2O_2. 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 H_2O_2 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 H_2O_2 as the mediator of FMD in patients with CAD.[37] 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 sensitive,[127] which strategically positions it for a role in shear-induced mitochondrial H_2O_2 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 H_2O_2, and reductions in ceramide levels can restore NO as the mediator of dilation in CAD.[128] 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.[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, catalytic subunit of telomerase stimulates NO production from NOS.[132] Mice deficient in the gene for catalytic subunit of telomerase have elevated levels of mitochondrial H_2O_2.[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, peroxisome proliferator-activated receptor γ, an inducer of telomerase, evokes the same restoration in the mechanism of dilation.[137] 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

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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 vasodilation[139] 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.[114,140,141] 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 adrenomedullin,[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.[144] 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 H_2O_2, an EDHF that opens BK_{Ca} 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 H_2O_2, 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 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 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 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, 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, 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 environment.[146] 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 (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 because human coronary arteriolar dilation to adenosine is not blocked by the selective K_{ATP} channel blocker, glibenclamide,[149–151] whereas dilation to hypoxia is either inhibited[152] 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 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 function[107] and affect the downstream microcirculation.[153] 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 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 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 et al,[154] bradykinin relaxes mesenteric arterioles in humans. The principal endothelium-derived mediator is H_2O_2, with a smaller contribution from gap junctions.[154] Although the source of H_2O_2 was not defined, the same group found in mouse mesenteric arterioles that H_2O_2 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 D_2.[156] The net response is a modest dilation. Prostaglandin D_2 plays no role

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in unaffected adjacent regions of bowel[156]; 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 al[157]). 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.[160] 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 arteriolar endothelium expresses functionally significant amounts of β-3-adrenoreceptors.[161] 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 ventricular microvessels from diseased hearts produce less NO to acetylcholine than vessels from nondiseased hearts.[162] 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 to 4.5 hours (average, 2.8) of in-hospital death.[163]

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] VSMCs have a membrane resistance of $>10^8$ ohms[166]; 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 >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, elicit dilation to β -adrenergic stimulation via cyclic adenosine monophosphate,[167] and participate in metabolic dilation.[168] (2) BK_{Ca} maintain E_m upon changes in intracellular Ca^{+2} levels acting as brakes for vasoconstrictor stimuli[169] and are a primary target for EDHF[55,170] 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 glucose[179] or superoxide[180] 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 hypercholesterolemia[183,184] and to pathophysiologically 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 animals[186,187] 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[189] with resulting dilation, but at higher cellular oxidative states, inhibition of dilation is seen.[190] These redox changes are particularly prominent in K_v 1.5 channels,[191,192] and their modulation also occurs in the human vasculature[193–195] where reversible sulfenic modification of cysteine residues are observed.[192] Oxidative conditions, such as atrial fibrillation, are associated with reduced K_v 1.5 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 Ca^{2+} and membrane depolarization.[197,198] These channels do not contribute to resting

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myogenic tone[199,200]; however, they are activated by a number of endothelium-derived relaxing factors, such as NO[201] 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 H_2O_2 near the sarcolemmal membrane.[205] 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[206] raises myogenic tone, which is countered by upregulation in expression of the α-subunit (pore forming) of BK_{Ca} channels in the aorta[207] and cerebral microvasculature[208] 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, BK_{Ca} smooth muscle activity is increased with disease. Wiecha et al[209] showed significantly higher activity of BK_{Ca} 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 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 because of H_2O_2.[210] This effect is mediated by oxidation of a key cysteine residue in the bowl region of the channel.[211] Other oxidants, such as peroxynitrite, impair activity of BK_{Ca} channels in VSMC of human coronary arterioles.[212] However, the situation is more complex in that the same oxidants (H_2O_2) can also enhance activity of BK_{Ca} channels in human VSMC as described above. It is unclear circumstances that dictate which action of H_2O_2 is observed. This speaks to the importance of H_2O_2 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 H_2O_2, activate BK_{Ca} in the underlying smooth muscle.[213] NO-mediated vasodilation involves phosphorylation of protein kinase G_1, which opens BK_{Ca} channels.[214] 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 H_2O_2 formation, as well as the amount produced and local antioxidant levels, determines the net effect on channel activity and vascular tone.

BK_{Ca} channels and K_v channels work together to mediate dilation to H_2O_2. Preliminary data from the Zhang lab show that in patients without CAD, H_2O_2 dilation depends on opening of K_v and BK_{Ca} channels.[215] 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 K_v 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 H_2O_2) but also affects the VSMC potassium channels that respond to H_2O_2. 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.

References

1. Bassingthwaighte JB, King RB, Roger SA. Fractal nature of regional myocardial blood flow heterogeneity. *Circ Res*. 1989;65:578–590.
2. Avakian A, Kalina RE, Sage EH, Rambhia AH, Elliott KE, Chuang EL, Clark JI, Hwang JN, Parsons-Wingerter P. Fractal analysis of region-based vascular change in the normal and non-proliferative diabetic retina. *Curr Eye Res*. 2002;24:274–280.
3. Nagaoka T, Yoshida A. Relationship between retinal fractal dimensions and retinal circulation in patients with type 2 diabetes mellitus. *Curr Eye Res*. 2013;38:1148–1152. doi: 10.3109/02713683.2013.805232.
4. Lanza GA, Crea F. Primary coronary microvascular dysfunction: clinical presentation, pathophysiology, and management. *Circulation*. 2010;121:2317–2325. doi: 10.1161/CIRCULATIONAHA.109.900191.
5. Brush JE Jr, Cannon RO 3rd, Schenke WH, Bonow RO, Leon MB, Maron BJ, Epstein SE. Angina due to coronary microvascular disease in hypertensive patients without left ventricular hypertrophy. *N Engl J Med*. 1988;319:1302–1307. doi: 10.1056/NEJM198811173192002.
6. Cannon RO III, Epstein SE. "Microvascular angina" as a cause of chest pain with angiographically normal coronary arteries. *Am J Cardiol*. 1988;61:1338–1343.
7. Cannon RO III, Camici PG, Epstein SE. Pathophysiological dilemma of syndrome x. *Circulation*. 1992;85:883–892.
8. den Uil CA, Lagrand WK, van der Ent M, Jewbali LS, Cheng JM, Spronk PE, Simoons ML. Impaired microcirculation predicts poor outcome of patients with acute myocardial infarction complicated by cardiogenic shock. *Eur Heart J*. 2010;31:3032–3039. doi: 10.1093/eurheartj/ehq324.
9. Halcox JP, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA, Nour KR, Quyyumi AA. Prognostic value of coronary vascular endothelial dysfunction. *Circulation*. 2002;106:653–658.
10. Cecchi F, Olivotto I, Gistri R, Lorenzoni R, Chiriatti G, Camici PG. Coronary microvascular dysfunction and prognosis in hypertrophic

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cardiomyopathy. *N Engl J Med*. 2003;349:1027–1035. doi: 10.1056/NEJMoa025050.
11. Mitchell GF, Parise H, Vita JA, Larson MG, Warner E, Keaney JF Jr, Keyes MJ, Levy D, Vasan RS, Benjamin EJ. Local shear stress and brachial artery flow-mediated dilation: the Framingham Heart Study. *Hypertension*. 2004;44:134–139. doi: 10.1161/01.HYP.0000137305.77635.68.
12. van de Hoef TP, van Lavieren MA, Damman P, Delewi R, Piek MA, Chamuleau SA, Voskuil M, Henriques JP, Koch KT, de Winter RJ, Spaan JA, Siebes M, Tijssen JG, Meuwissen M, Piek JJ. Physiological basis and long-term clinical outcome of discordance between fractional flow reserve and coronary flow velocity reserve in coronary stenoses of intermediate severity. *Circ Cardiovasc Interv*. 2014;7:301–311. doi: 10.1161/CIRCINTERVENTIONS.113.001049.
13. Taqueti VR, Everett BM, Murthy VL, Gaber M, Foster CR, Hainer J, Blankstein R, Dorbala S, Di Carli MF. Interaction of impaired coronary flow reserve and cardiomyocyte injury on adverse cardiovascular outcomes in patients without overt coronary artery disease. *Circulation*. 2015;131:528–535. doi: 10.1161/CIRCULATIONAHA.114.009716.
14. Murthy VL, Naya M, Taqueti VR, Foster CR, Gaber M, Hainer J, Dorbala S, Blankstein R, Rimoldi O, Camici PG, Di Carli MF. Effects of sex on coronary microvascular dysfunction and cardiac outcomes. *Circulation*. 2014;129:2518–2527. doi: 10.1161/CIRCULATIONAHA.113.008507.
15. van Kranenburg M, Magro M, Thiele H, et al. Prognostic value of microvascular obstruction and infarct size, as measured by CMR in STEMI patients. *JACC Cardiovasc Imaging*. 2014;7:930–939. doi: 10.1016/j.jcmg.2014.05.010.
16. Rizzoni D, Porteri E, De Ciuceis C, Boari GE, Zani F, Miclini M, Paiardi S, Tiberio GA, Giulini SM, Muiesan ML, Castellano M, Rosei EA. Lack of prognostic role of endothelial dysfunction in subcutaneous small resistance arteries of hypertensive patients. *J Hypertens*. 2006;24:867–873. doi: 10.1097/01.hjh.0000222756.76982.53.
17. Nahser PJ Jr, Brown RE, Oskarsson H, Winniford MD, Rossen JD. Maximal coronary flow reserve and metabolic coronary vasodilation in patients with diabetes mellitus. *Circulation*. 1995;91:635–640.
18. Di Carli MF, Janisse J, Grunberger G, Ager J. Role of chronic hyperglycemia in the pathogenesis of coronary microvascular dysfunction in diabetes. *J Am Coll Cardiol*. 2003;41:1387–1393.
19. Chan W, Stub D, Clark DJ, Ajani AE, Andrianopoulos N, Brennan AL, New G, Black A, Shaw JA, Reid CM, Dart AM, Duffy SJ; Melbourne Interventional Group Investigators. Usefulness of transient and persistent no reflow to predict adverse clinical outcomes following percutaneous coronary intervention. *Am J Cardiol*. 2012;109:478–485. doi: 10.1016/j.amjcard.2011.09.037.
20. Fabregat-Andrés Ó, Cubillos A, Ferrando-Beltrán M, Bochard-Villanueva B, Estornell-Erill J, Fácila L, Ridocci-Soriano F, Morell S. Mean platelet volume is associated with infarct size and microvascular obstruction estimated by cardiac magnetic resonance in ST segment elevation myocardial infarction. *Blood Coagul Fibrinolysis*. 2013;24:424–427. doi: 10.1097/MBC.0b013e32835d9bca.
21. Kloner RA, Ganote CE, Jennings RB. The "no-reflow" phenomenon after temporary coronary occlusion in the dog. *J Clin Invest*. 1974;54:1496–1508. doi: 10.1172/JCI107898.
22. Robbers LF, Eerenberg ES, Teunissen PF, Jansen MF, Hollander MR, Horrevoets AJ, Knaapen P, Nijveldt R, Heymans MW, Levi MM, van Rossum AC, Niessen HW, Marcu CB, Beek AM, van Royen N. Magnetic resonance imaging-defined areas of microvascular obstruction after acute myocardial infarction represent microvascular destruction and haemorrhage. *Eur Heart J*. 2013;34:2346–2353. doi: 10.1093/eurheartj/eht100.
23. Carrick D, Oldroyd KG, McEntegart M, et al. A randomized trial of deferred stenting versus immediate stenting to prevent no- or slow-reflow in acute ST-segment elevation myocardial infarction (DEFER-STEMI). *J Am Coll Cardiol*. 2014;63:2088–2098. doi: 10.1016/j.jacc.2014.02.530.
24. Zeiher AM, Drexler H, Wollschläger H, Just H. Endothelial dysfunction of the coronary microvasculature is associated with coronary blood flow regulation in patients with early atherosclerosis. *Circulation*. 1991;84:1984–1992.
25. Gilligan DM, Guetta V, Panza JA, García CE, Quyyumi AA, Cannon RO 3rd. Selective loss of microvascular endothelial function in human hypercholesterolemia. *Circulation*. 1994;90:35–41.
26. Rubinshtein R, Yang EH, Rihal CS, Prasad A, Lennon RJ, Best PJ, Lerman LO, Lerman A. Coronary microcirculatory vasodilator function in relation to risk factors among patients without obstructive coronary disease and low to intermediate Framingham score. *Eur Heart J*. 2010;31:936–942. doi: 10.1093/eurheartj/ehp459.
27. Zeiher AM, Krause T, Schächinger V, Minners J, Moser E. Impaired endothelium-dependent vasodilation of coronary resistance vessels is associated with exercise-induced myocardial ischemia. *Circulation*. 1995;91:2345–2352.
28. Reis SE, Holubkov R, Conrad Smith AJ, Kelsey SF, Sharaf BL, Reichek N, Rogers WJ, Merz CN, Sopko G, Pepine CJ; WISE Investigators. Coronary microvascular dysfunction is highly prevalent in women with chest pain in the absence of coronary artery disease: results from the NHLBI WISE study. *Am Heart J*. 2001;141:735–741.
29. von Mering GO, Arant CB, Wessel TR, McGorray SP, Bairey Merz CN, Sharaf BL, Smith KM, Olson MB, Johnson BD, Sopko G, Handberg E, Pepine CJ, Kerensky RA; National Heart, Lung, and Blood Institute. Abnormal coronary vasomotion as a prognostic indicator of cardiovascular events in women: results from the National Heart, Lung, and Blood Institute-Sponsored Women's Ischemia Syndrome Evaluation (WISE). *Circulation*. 2004;109:722–725. doi: 10.1161/01.CIR.0000115525.92645.16.
30. Pepine CJ, Anderson RD, Sharaf BL, Reis SE, Smith KM, Handberg EM, Johnson BD, Sopko G, Bairey Merz CN. Coronary microvascular reactivity to adenosine predicts adverse outcome in women evaluated for suspected ischemia results from the National Heart, Lung and Blood Institute WISE (Women's Ischemia Syndrome Evaluation) study. *J Am Coll Cardiol*. 2010;55:2825–2832. doi: 10.1016/j.jacc.2010.01.054.
31. Scalia R. The microcirculation in adipose tissue inflammation. *Rev Endocr Metab Disord*. 2013;14:69–76. doi: 10.1007/s11154-013-9236-x.
32. Patt BT, Jarjoura D, Haddad DN, Sen CK, Roy S, Flavahan NA, Khayat RN. Endothelial dysfunction in the microcirculation of patients with obstructive sleep apnea. *Am J Respir Crit Care Med*. 2010;182:1540–1545. doi: 10.1164/rccm.201002-0162OC.
33. Rigo F, Sicari R, Citro R, Ossena G, Buja P, Picano E. Diffuse, marked, reversible impairment in coronary microcirculation in stress cardiomyopathy: a Doppler transthoracic echo study. *Ann Med*. 2009;41:462–470. doi: 10.1080/07853890903022793.
34. Treasure CB, Vita JA, Cox DA, Fish RD, Gordon JB, Mudge GH, Colucci WS, Sutton MG, Selwyn AP, Alexander RW. Endothelium-dependent dilation of the coronary microvasculature is impaired in dilated cardiomyopathy. *Circulation*. 1990;81:772–779.
35. Laguens R, Alvarez P, Vigliano C, Cabeza Meckert P, Favaloro L, Diez M, Favaloro R. Coronary microcirculation remodeling in patients with idiopathic dilated cardiomyopathy. *Cardiology*. 2011;119:191–196. doi: 10.1159/000331440.
36. Prasad A, Higano ST, Al Suwaidi J, Holmes DR Jr, Mathew V, Pumper G, Lennon RJ, Lerman A. Abnormal coronary microvascular endothelial function in humans with asymptomatic left ventricular dysfunction. *Am Heart J*. 2003;146:549–554. doi: 10.1016/S0002-8703(03)00364-8.
37. Paulus WJ, Tschöpe C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. *J Am Coll Cardiol*. 2013;62:263–271. doi: 10.1016/j.jacc.2013.02.092.
38. Deban L, Correale C, Vetrano S, Malesci A, Danese S. Multiple pathogenic roles of microvasculature in inflammatory bowel disease: a Jack of all trades. *Am J Pathol*. 2008;172:1457–1466. doi: 10.2353/ajpath.2008.070593.
39. Hatoum OA, Gauthier KM, Binion DG, Miura H, Telford G, Otterson MF, Campbell WB, Gutterman DD. Novel mechanism of vasodilation in inflammatory bowel disease. *Arterioscler Thromb Vasc Biol*. 2005;25:2355–2361. doi: 10.1161/01.ATV.0000184757.50141.8d.
40. Moises HW, Wollschläger D, Binder H. Functional genomics indicate that schizophrenia may be an adult vascular-ischemic disorder. *Transl Psychiatry*. 2015;5:e616. doi: 10.1038/tp.2015.103.
41. Migrino RQ, Hari P, Gutterman DD, Bright M, Truran S, Schlundt B, Phillips SA. Systemic and microvascular oxidative stress induced by light chain amyloidosis. *Int J Cardiol*. 2010;145:67–68. doi: 10.1016/j.ijcard.2009.04.044.
42. Miura H, Toyama K, Pratt PF, Gutterman DD. Cigarette smoking impairs Na+-K+-ATPase activity in the human coronary microcirculation. *Am J Physiol Heart Circ Physiol*. 2011;300:H109–H117. doi: 10.1152/ajpheart.00237.2010.
43. Holowatz LA. Human cutaneous microvascular ageing: potential insights into underlying physiological mechanisms of endothelial function and dysfunction. *J Physiol*. 2008;586:3301. doi: 10.1113/jphysiol.2008.157594.
44. Wright SA, O'Prey FM, Rea DJ, Plumb RD, Gamble AJ, Leahey WJ, Devine AB, McGivern RC, Johnston DG, Finch MB, Bell AL, McVeigh GE. Microcirculatory hemodynamics and endothelial dysfunction in systemic lupus erythematosus. *Arterioscler Thromb Vasc Biol*. 2006;26:2281–2287. doi: 10.1161/01.ATV.0000238351.82900.7f.

Downloaded from http://ahajournals.org by on March 28, 2019

45. Thijssen DH, Green DJ, Hopman MT. Blood vessel remodeling and physical inactivity in humans. *J Appl Physiol (1985)*. 2011;111:1836–1845. doi: 10.1152/japplphysiol.00394.2011.
46. Feher A, Chen SY, Bagi Z, Arora V. Prevention and treatment of no-reflow phenomenon by targeting the coronary microcirculation. *Rev Cardiovasc Med*. 2014;15:38–51.
47. Feng J, Liu Y, Clements RT, Sodha NR, Khabbaz KR, Senthilnathan V, Nishimura KK, Alper SL, Sellke FW. Calcium-activated potassium channels contribute to human coronary microvascular dysfunction after cardioplegic arrest. *Circulation*. 2008;118:S46–S51. doi: 10.1161/CIRCULATIONAHA.107.755827.
48. Mohri M, Shimokawa H, Hirakawa Y, Masumoto A, Takeshita A. Rho-kinase inhibition with intracoronary fasudil prevents myocardial ischemia in patients with coronary microvascular spasm. *J Am Coll Cardiol*. 2003;41:15–19.
49. Naraoka M, Matsuda N, Shimamura N, Asano K, Ohkuma H. The role of arterioles and the microcirculation in the development of vasospasm after aneurysmal SAH. *Biomed Res Int*. 2014;2014:253746. doi: 10.1155/2014/253746.
50. Medhora M, Daniels J, Mundey K, Fisslthaler B, Busse R, Jacobs ER, Harder DR. Epoxygenase-driven angiogenesis in human lung microvascular endothelial cells. *Am J Physiol Heart Circ Physiol*. 2003;284:H215–H224. doi: 10.1152/ajpheart.01118.2001.
51. Datla SR, Peshavariya H, Dusting GJ, Mahadev K, Goldstein BJ, Jiang F. Important role of Nox4 type NADPH oxidase in angiogenic responses in human microvascular endothelial cells in vitro. *Arterioscler Thromb Vasc Biol*. 2007;27:2319–2324. doi: 10.1161/ATVBAHA.107.149450.
52. Grgic I, Eichler I, Heinau P, Si H, Brakemeier S, Hoyer J, Köhler R. Selective blockade of the intermediate-conductance Ca2+-activated K+ channel suppresses proliferation of microvascular and macrovascular endothelial cells and angiogenesis in vivo. *Arterioscler Thromb Vasc Biol*. 2005;25:704–709. doi: 10.1161/01.ATV.0000156399.12787.5c.
53. Durand MJ, Phillips SA, Widlansky ME, Otterson MF, Gutterman DD. The vascular renin-angiotensin system contributes to blunted vasodilation induced by transient high pressure in human adipose microvessels. *Am J Physiol Heart Circ Physiol*. 2014;307:H25–H32. doi: 10.1152/ajpheart.00055.2014.
54. Paraskevaidis IA, Iliodromitis EK, Ikonomidis I, Rallidis L, Hamodraka E, Parissis J, Andoniadis A, Tzortzis S, Anastasiou-Nana M. The effect of acute administration of statins on coronary microcirculation during the pre-revascularization period in patients with myocardial infraction. *Atherosclerosis*. 2012;223:184–189. doi: 10.1016/j.atherosclerosis.2012.04.002.
55. Larsen BT, Miura H, Hatoum OA, Campbell WB, Hammock BD, Zeldin DC, Falck JR, Gutterman DD. Epoxyeicosatrienoic and dihydroxyeicosatrienoic acids dilate human coronary arterioles via BK(Ca) channels: implications for soluble epoxide hydrolase inhibition. *Am J Physiol Heart Circ Physiol*. 2006;290:H491–H499. doi: 10.1152/ajpheart.00927.2005.
56. Dhanasekaran A, Al-Saghir R, Lopez B, Zhu D, Gutterman DD, Jacobs ER, Medhora M. Protective effects of epoxyeicosatrienoic acids on human endothelial cells from the pulmonary and coronary vasculature. *Am J Physiol Heart Circ Physiol*. 2006;291:H517–H531. doi: 10.1152/ajpheart.00953.2005.
57. Tacconelli S, Patrignani P. Inside epoxyeicosatrienoic acids and cardiovascular disease. *Front Pharmacol*. 2014;5:239. doi: 10.3389/fphar.2014.00239.
57a. Yoshino S, Cilluffo R, Best PJ, Atkinson EJ, Aoki T, Cunningham JM, de Andrade M, Choi BJ, Lerman LO, Lerman A. Single nucleotide polymorphisms associated with abnormal coronary microvascular function. *Coron Artery Dis*. 2014;25:281-289. doi: 10.1097/MCA.0000000000000104.
58. Fedele F, Mancone M, Chilian WM, Severino P, Canali E, Logan S, De Marchis ML, Volterrani M, Palmirotta R, Guadagni F. Role of genetic polymorphisms of ion channels in the pathophysiology of coronary microvascular dysfunction and ischemic heart disease. *Basic Res Cardiol*. 2013;108:387. doi: 10.1007/s00395-013-0387-4.
59. Mashiba J, Koike G, Kamiunten H, Ikeda M, Sunagawa K. Vasospastic angina and microvascular angina are differentially influenced by PON1 A632G polymorphism in the Japanese. *Circ J*. 2005;69:1466–1471.
60. Ekmekci A, Güngör B, Özcan KS, Abaci N, Ilhan E, Ekmekci SS, Kemaloğlu T, Osmonov D, Ustek D, Eren M. Evaluation of coronary microvascular function and nitric oxide synthase intron 4a/b polymorphism in patients with coronary slow flow. *Coron Artery Dis*. 2013;24:461–467. doi: 10.1097/MCA.0b013e328363258c.
61. Katoh A, Ikeda H, Takajo Y, Haramaki N, Murohara T, Shintani S, Kanaya S, Yokoyama S, Ueno T, Honma T, Imaizumi T. Coexistence of impairment of endothelium-derived nitric oxide and platelet-derived nitric oxide in patients with coronary risk factors. *Circ J*. 2002;66:837–840.
62. Al-Fiadh AH, Farouque O, Kawasaki R, Nguyen TT, Uddin N, Freeman M, Patel SK, Burrell LM, Wong TY. Retinal microvascular structure and function in patients with risk factors of atherosclerosis and coronary artery disease. *Atherosclerosis*. 2014;233:478–484. doi: 10.1016/j.atherosclerosis.2013.12.044.
63. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, deLaGrange D, Lieberman EH, Ganz P, Creager MA, Yeung AC, Selwyn AP. Close relation of endothelial function in the human coronary and peripheral circulations. *JAmCollCardiol*. 1995;26:1235–1241.
64. Kunadian V, Harrigan C, Zorkun C, Palmer AM, Ogando KJ, Biller LH, Lord EE, Williams SP, Lew ME, Ciaglo LN, Buros JL, Marble SJ, Gibson WJ, Gibson CM. Use of the TIMI frame count in the assessment of coronary artery blood flow and microvascular function over the past 15 years. *J Thromb Thrombolysis*. 2009;27:316–328. doi: 10.1007/s11239-008-0220-3.
65. Hamada S, Nishiue T, Nakamura S, Sugiura T, Kamihata H, Miyoshi H, Imuro Y, Iwasaka T. TIMI frame count immediately after primary coronary angioplasty as a predictor of functional recovery in patients with TIMI 3 reperfused acute myocardial infarction. *J Am Coll Cardiol*. 2001;38:666–671.
66. Gibson CM, Murphy SA, Rizzo MJ, Ryan KA, Marble SJ, McCabe CH, Cannon CP, Van de Werf F, Braunwald E. Relationship between TIMI frame count and clinical outcomes after thrombolytic administration. Thrombolysis In Myocardial Infarction (TIMI) Study Group. *Circulation*. 1999;99:1945–1950.
67. Strain WD, Adingupu DD, Shore AC. Microcirculation on a large scale: techniques, tactics and relevance of studying the microcirculation in larger population samples. *Microcirculation*. 2012;19:37–46. doi: 10.1111/j.1549-8719.2011.00140.x.
68. Wei K, Lindner J. Contrast ultrasound in the assessment of patients presenting with suspected cardiac ischemia. *Crit Care Med*. 2007;35:S280–S289. doi: 10.1097/01.CCM.0000260678.03628.4C.
69. Miller FJ Jr, Dellsperger KC, Gutterman DD. Myogenic constriction of human coronary arterioles. *Am J Physiol*. 1997;273:H257–H264.
70. Cohen MV, Kirk ES. Differential response of large and small coronary arteries to nitroglycerin and angiotensin. *CircRes*. 1973;18:445–453.
71. Penny WF, Rockman H, Long J, Bhargava V, Carrigan K, Ibriham A, Shabetai R, Ross J Jr, Peterson KL. Heterogeneity of vasomotor response to acetylcholine along the human coronary artery. *J Am Coll Cardiol*. 1995;25:1046–1055.
72. Kuo L, Davis MJ, Chilian WM. Endothelium-dependent, flow-induced dilation of isolated coronary arterioles. *Am J Physiol*. 1990;259:H1063–H1070.
73. Koller A, Kaley G. Prostaglandins mediate arteriolar dilation to increased blood flow velocity in skeletal muscle microcirculation. *Circ Res*. 1990;67:529–534.
74. Huang A, Sun D, Carroll MA, Jiang H, Smith CJ, Connetta JA, Falck JR, Shesely EG, Koller A, Kaley G. EDHF mediates flow-induced dilation in skeletal muscle arterioles of female eNOS-KO mice. *Am J Physiol Heart Circ Physiol*. 2001;280:H2462–H2469.
75. Myers PR, Banitt PF, Guerra R Jr, Harrison DG. Role of the endothelium in modulation of the acetylcholine vasoconstrictor response in porcine coronary microvessels. *Cardiovasc Res*. 1991;25:129–137.
76. Brunori M, Giuffrè A, Sarti P, Stubauer G, Wilson MT. Nitric oxide and cellular respiration. *Cell Mol Life Sci*. 1999;56:549–557.
77. Brookes PS, Kraus DW, Shiva S, Doeller JE, Barone MC, Patel RP, Lancaster JR Jr, Darley-Usmar V. Control of mitochondrial respiration by NO*, effects of low oxygen and respiratory state. *J Biol Chem*. 2003;278:31603–31609. doi: 10.1074/jbc.M211784200.
78. Yao SK, Ober JC, Krishnaswami A, Ferguson JJ, Anderson HV, Golino P, Buja LM, Willerson JT. Endogenous nitric oxide protects against platelet aggregation and cyclic flow variations in stenosed and endothelium-injured arteries. *Circulation*. 1992;86:1302–1309.
79. Broeders MA, Tangelder GJ, Slaaf DW, Reneman RS, oude Egbrink MG. Endogenous nitric oxide protects against thromboembolism in venules but not in arterioles. *Arterioscler Thromb Vasc Biol*.. 1998;18:139–45.
80. Noireaud J, Andriantsitohaina R. Recent insights in the paracrine modulation of cardiomyocyte contractility by cardiac endothelial cells. *Biomed Res Int*. 2014;2014:923805. doi: 10.1155/2014/923805.
81. Namekata I, Fujiki S, Kawakami Y, Moriwaki R, Takeda K, Kawanishi T, Takahara A, Shigenobu K, Tanaka H. Intracellular mechanisms and

Downloaded from http://ahajournals.org by on March 28, 2019

receptor types for endothelin-1-induced positive and negative inotropy in mouse ventricular myocardium. *Naunyn Schmiedebergs Arch Pharmacol*. 2008;376:385–395. doi: 10.1007/s00210-007-0228-9.
82. Harder DR, Campbell WB and Roman RJ. Role of cytochrome P-450 enzymes and metabolites of arachidonic acid in the control of vascular tone. *J Vasc Res*. 1995;32:79–92.
83. Larsen BT, Bubolz AH, Mendoza SA, Pritchard KA Jr, Gutterman DD. Bradykinin-induced dilation of human coronary arterioles requires NADPH oxidase-derived reactive oxygen species. *Arterioscler Thromb Vasc Biol*. 2009;29:739–745. doi: 10.1161/ATVBAHA.108.169367.
84. Miura H, Wachtel RE, Liu Y, Loberiza FR Jr, Saito T, Miura M, Gutterman DD. Flow-induced dilation of human coronary arterioles: important role of Ca(2+)-activated K(+) channels. *Circulation*. 2001;103:1992–1998.
85. Ungvari Z, Orosz Z, Labinskyy N, Rivera A, Xiangmin Z, Smith K, Csiszar A. Increased mitochondrial H_2O_2 production promotes endothelial NF-kappaB activation in aged rat arteries. *Am J Physiol Heart Circ Physiol*. 2007;293:H37–H47. doi: 10.1152/ajpheart.01346.2006.
86. Bychkov R, Pieper K, Ried C, Milosheva M, Bychkov E, Luft FC, Haller H. Hydrogen peroxide, potassium currents, and membrane potential in human endothelial cells. *Circulation*. 1999;99:1719–1725.
87. Ody C, Junod AF. Effect of variable glutathione peroxidase activity on H2O2-related cytotoxicity in cultured aortic endothelial cells. *Proc Soc Exp Biol Med*. 1985;180:103–111.
88. Witting PK, Rayner BS, Wu BJ, Ellis NA, Stocker R. Hydrogen peroxide promotes endothelial dysfunction by stimulating multiple sources of superoxide anion radical production and decreasing nitric oxide bioavailability. *Cell Physiol Biochem*. 2007;20:255–268. doi: 10.1159/000107512.
89. Talukdar S, Oh da Y, Bandyopadhyay G, Li D, Xu J, McNelis J, Lu M, Li P, Yan Q, Zhu Y, Ofrecio J, Lin M, Brenner MB, Olefsky JM. Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. *Nat Med*. 2012;18:1407–1412. doi: 10.1038/nm.2885.
90. Halberg N, Khan T, Trujillo ME, Wernstedt-Asterholm I, Attie AD, Sherwani S, Wang ZV, Landskroner-Eiger S, Dineen S, Magalang UJ, Brekken RA, Scherer PE. Hypoxia-inducible factor 1alpha induces fibrosis and insulin resistance in white adipose tissue. *Mol Cell Biol*. 2009;29:4467–4483. doi: 10.1128/MCB.00192-09.
91. Westermann D, Lindner D, Kasner M, Zietsch C, Savvatis K, Escher F, von Schlippenbach J, Skurk C, Steendijk P, Riad A, Poller W, Schultheiss HP, Tschöpe C. Cardiac inflammation contributes to changes in the extracellular matrix in patients with heart failure and normal ejection fraction. *Circ Heart Fail*. 2011;4:44–52. doi: 10.1161/CIRCHEARTFAILURE.109.931451.
92. Tschöpe C, Bock CT, Kasner M, Noutsias M, Westermann D, Schwimmbeck PL, Pauschinger M, Poller WC, Kühl U, Kandolf R, Schultheiss HP. High prevalence of cardiac parvovirus B19 infection in patients with isolated left ventricular diastolic dysfunction. *Circulation*. 2005;111:879–886. doi: 10.1161/01.CIR.0000155615.68924.B3.
93. Hugel B, Martínez MC, Kunzelmann C, Freyssinet JM. Membrane microparticles: two sides of the coin. *Physiology (Bethesda)*. 2005;20:22–27. doi: 10.1152/physiol.00029.2004.
94. Dignat-George F, Boulanger CM. The many faces of endothelial microparticles. *Arterioscler Thromb Vasc Biol*. 2011;31:27–33. doi: 10.1161/ATVBAHA.110.218123.
95. Boulanger CM, Scoazec A, Ebrahimian T, Henry P, Mathieu E, Tedgui A, Mallat Z. Circulating microparticles from patients with myocardial infarction cause endothelial dysfunction. *Circulation*. 2001;104:2649–2652.
96. Zernecke A, Bidzhekov K, Noels H, Shagdarsuren E, Gan L, Denecke B, Hristov M, Köppel T, Jahantigh MN, Lutgens E, Wang S, Olson EN, Schober A, Weber C. Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. *Sci Signal*. 2009;2:ra81. doi: 10.1126/scisignal.2000610.
97. Seddon MD, Chowienczyk PJ, Brett SE, Casadei B, Shah AM. Neuronal nitric oxide synthase regulates basal microvascular tone in humans in vivo. *Circulation*. 2008;117:1991–1996. doi: 10.1161/CIRCULATIONAHA.107.744540.
98. Melikian N, Seddon MD, Casadei B, Chowienczyk PJ, Shah AM. Neuronal nitric oxide synthase and human vascular regulation. *Trends Cardiovasc Med*. 2009;19:256–262. doi: 10.1016/j.tcm.2010.02.007.
99. Seddon M, Melikian N, Dworakowski R, Shabeeh H, Jiang B, Byrne J, Casadei B, Chowienczyk P, Shah AM. Effects of neuronal nitric oxide synthase on human coronary artery diameter and blood flow in vivo. *Circulation*. 2009;119:2656–2662. doi: 10.1161/CIRCULATIONAHA.108.822205.
100. Lamping KG, Nuno DW, Faraci FM. Role of nNOS in dilation to acetylcholine in coronary arteries from eNOS-deficient mice. *Faseb J*. 1999:A1085.
101. Huang A, Sun D, Shesely EG, Levee EM, Koller A, Kaley G. Neuronal NOS-dependent dilation to flow in coronary arteries of male eNOS-KO mice. *Am J Physiol Heart Circ Physiol*. 2002;282:H429–H436. doi: 10.1152/ajpheart.00501.2001.
102. Fischer D, Landmesser U, Spiekermann S, Hilfiker-Kleiner D, Hospely M, Müller M, Busse R, Fleming I, Drexler H. Cytochrome P450 2C9 is involved in flow-dependent vasodilation of peripheral conduit arteries in healthy subjects and in patients with chronic heart failure. *Eur J Heart Fail*. 2007;9:770–775. doi: 10.1016/j.ejheart.2007.05.005.
103. Gokce N, Keaney JF Jr, Hunter LM, Watkins MT, Nedeljkovic ZS, Menzoian JO, Vita JA. Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. *J Am Coll Cardiol*. 2003;41:1769–1775.
104. Patti G, Pasceri V, Melfi R, Goffredo C, Chello M, D'Ambrosio A, Montesanti R, Di Sciascio G. Impaired flow-mediated dilation and risk of restenosis in patients undergoing coronary stent implantation. *Circulation*. 2005;111:70–75. doi: 10.1161/01.CIR.0000151308.06673.D2.
105. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Münzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation*. 2001;104:2673–2678.
106. Schächinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation*. 2000;101:1899–1906.
107. Jurva JW, Phillips SA, Syed AQ, Syed AY, Pitt S, Weaver A, Gutterman DD. The effect of exertional hypertension evoked by weight lifting on vascular endothelial function. *J Am Coll Cardiol*. 2006;48:588–589. doi: 10.1016/j.jacc.2006.05.004.
108. Title LM, Cummings PM, Giddens K, Nassar BA. Oral glucose loading acutely attenuates endothelium-dependent vasodilation in healthy adults without diabetes: an effect prevented by vitamins C and E. *J Am Coll Cardiol*. 2000;36:2185–2191.
109. Dharmashankar K, Welsh A, Wang J, Kizhakekuttu TJ, Ying R, Gutterman DD, Widlansky ME. Nitric oxide synthase-dependent vasodilation of human subcutaneous arterioles correlates with noninvasive measurements of endothelial function. *Am J Hypertens*. 2012;25:528–534. doi: 10.1038/ajh.2012.8.
110. Cooper A, Heagerty AM. Endothelial dysfunction in human intramyocardial small arteries in atherosclerosis and hypercholesterolemia. *Am J Physiol*. 1998;275:H1482–H1488.
111. Miura H, Liu Y, Gutterman DD. Human coronary arteriolar dilation to bradykinin depends on membrane hyperpolarization. *Circulation*. 1999;99:3132–3138.
112. Kichuk MR, Zhang X, Oz M, Michler R, Kaley G, Nasjletti A, Hintze TH. Angiotensin-converting enzyme inhibitors promote nitric oxide production in coronary microvessels from failing explanted human hearts. *Am J Cardiol*. 1997;80:137A–142A.
113. Tiefenbacher CP, Friedrich S, Bleeke T, Vahl C, Chen X, Niroomand F. ACE inhibitors and statins acutely improve endothelial dysfunction of human coronary arterioles. *Am J Physiol Heart Circ Physiol*. 2004;286:H1425–H1432. doi: 10.1152/ajpheart.00783.2003.
114. Tiefenbacher CP, Bleeke T, Vahl C, Amann K, Vogt A, Kubler W. Endothelial dysfunction of coronary resistance arteries is improved by tetrahydrobiopterin in atherosclerosis. *Circulation*. 2000;102:2172–2179.
115. Matoba T, Shimokawa H, Nakashima M, Hirakawa Y, Mukai Y, Hirano K, Kanaide H, Takeshita A. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. *J Clin Invest*. 2000;106:1521–1530. doi: 10.1172/JCI10506.
116. Zinkevich NS, Wittenburg AL, Gutterman DD. Role of cyclooxygenase in flow-induced dilation of human coronary arterioles depends upone age. *Circulation*. 2010;122.
117. Miura H, Qiang C, Liu Y, Saito T, Miura M, Gutterman DD. Flow-mediated dilation of human coronary arterioles involves production of hydrogen peroxide. *Circulation*. 2001;104:II-285–II-286.
118. Liu Y, Zhao H, Li H, Kalyanaraman B, Nicolosi AC, Gutterman DD. Mitochondrial sources of H2O2 generation play a key role in flow-mediated dilation in human coronary resistance arteries. *Circ Res*. 2003;93:573–580. doi: 10.1161/01.RES.0000091261.19387.AE.
119. Burgoyne JR, Oka S, Ale-Agha N, Eaton P. Hydrogen peroxide sensing and signaling by protein kinases in the cardiovascular system. *Antioxid Redox Signal*. 2013;18:1042–1052. doi: 10.1089/ars.2012.4817.
120. Bubolz AH, Mendoza SA, Zheng X, Zinkevich NS, Li R, Gutterman DD, Zhang DX. Activation of endothelial TRPV4 channels mediates

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flow-induced dilation in human coronary arterioles: role of Ca2+ entry and mitochondrial ROS signaling. *Am J Physiol Heart Circ Physiol*. 2012;302:H634–H642. doi: 10.1152/ajpheart.00717.2011.
121. Liu Y, Li H, Bubolz AH, Zhang DX, Gutterman DD. Endothelial cytoskeletal elements are critical for flow-mediated dilation in human coronary arterioles. *Med Biol Eng Comput*. 2008;46:469–478. doi: 10.1007/s11517-008-0331-1.
122. Kurek K, Piotrowska DM, Wiesiolek-Kurek P, Chabowska A, Lukaszuk B, Zendzian-Piotrowska M. The role of sphingolipids in selected cardiovascular diseases. *Postepy Hig Med Dosw (Online)*. 2013;67:1018–26.
123. Li X, Becker KA, Zhang Y. Ceramide in redox signaling and cardiovascular diseases. *Cell Physiol Biochem*. 2010;26:41–48. doi: 10.1159/000315104.
124. Bismuth J, Lin P, Yao Q, Chen C. Ceramide: a common pathway for atherosclerosis? *Atherosclerosis*. 2008;196:497–504. doi: 10.1016/j.atherosclerosis.2007.09.018.
125. Deshpande SS, Qi B, Park YC, Irani K. Constitutive activation of rac1 results in mitochondrial oxidative stress and induces premature endothelial cell senescence. *Arterioscler Thromb Vasc Biol*. 2003;23:e1–e6.
126. Corda S, Laplace C, Vicaut E, Duranteau J. Rapid reactive oxygen species production by mitochondria in endothelial cells exposed to tumor necrosis factor-alpha is mediated by ceramide. *Am J Respir Cell Mol Biol*. 2001;24:762–768. doi: 10.1165/ajrcmb.24.6.4228.
127. Czarny M, Liu J, Oh P, Schnitzer JE. Transient mechanoactivation of neutral sphingomyelinase in caveolae to generate ceramide. *J Biol Chem*. 2003;278:4424–4430. doi: 10.1074/jbc.M210375200.
128. Freed JK, Beyer AM, LoGiudice JA, Hockenberry JC, Gutterman DD. Ceramide changes the mediator of flow-induced vasodilation from nitric oxide to hydrogen peroxide in the human microcirculation. *Circ Res*. 2014;115:525–532. doi: 10.1161/CIRCRESAHA.115.303881.
129. Wooten-Blanks LG, Song P, Senkal CE, Ogretmen B. Mechanisms of ceramide-mediated repression of the human telomerase reverse transcriptase promoter via deacetylation of Sp3 by histone deacetylase 1. *FASEB J*. 2007;21:3386–3397. doi: 10.1096/fj.07-8621com.
130. Santos JH, Meyer JN, Van Houten B. Mitochondrial localization of telomerase as a determinant for hydrogen peroxide-induced mitochondrial DNA damage and apoptosis. *Hum Mol Genet*. 2006;15:1757–1768. doi: 10.1093/hmg/ddl098.
131. Haendeler J, Dröse S, Büchner N, Jakob S, Altschmied J, Goy C, Spyridopoulos I, Zeiher AM, Brandt U, Dimmeler S. Mitochondrial telomerase reverse transcriptase binds to and protects mitochondrial DNA and function from damage. *Arterioscler Thromb Vasc Biol*. 2009;29:929–935. doi: 10.1161/ATVBAHA.109.185546.
132. Matsushita H, Chang E, Glassford AJ, Cooke JP, Chiu CP, Tsao PS. eNOS activity is reduced in senescent human endothelial cells: Preservation by hTERT immortalization. *Circ Res*. 2001;89:793–798.
133. Sahin E, Depinho RA. Linking functional decline of telomeres, mitochondria and stem cells during ageing. *Nature*. 2010;464:520–528. doi: 10.1038/nature08982.
134. Sahin E, Colla S, Liesa M, et al. Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature*. 2011;470:359–365. doi: 10.1038/nature09787.
135. Santos J, Gutterman DD, Beyer AM. Mitochondrial telomerase regulates flow mediated dilation by suppressing mitochondrial derived free radical production. *FASEB J*. 2014;28:664.1.
136. Eitan E, Tichon A, Gazit A, Gitler D, Slavin S, Priel E. Novel telomerase-increasing compound in mouse brain delays the onset of amyotrophic lateral sclerosis. *EMBO Mol Med*. 2012;4:313–329. doi: 10.1002/emmm.201200212.
137. Beyer AM, Gutterman DD. Activation of PPAR {gamma} converts the mechanism of flow-mediated dilation in human microvessels from H2O2 to NO by a telomerase dependent mechanism. *Circulation*. 2012;126:A9240.
138. Myers PR, Banitt PF, Guerra R Jr, Harrison DG. Characteristics of canine coronary resistance arteries: importance of endothelium. *Am J Physiol*. 1989;257:H603–H610.
139. Broten TP, Miyashiro JK, Moncada S, Feigl EO. Role of endothelium-derived relaxing factor in parasympathetic coronary vasodilation. *Am J Physiol*. 1992;262:H1579–H1584.
140. Miller FJ Jr, Dellsperger KC, Gutterman DD. Pharmacologic activation of the human coronary microcirculation in vitro: endothelium-dependent dilation and differential responses to acetylcholine. *Cardiovasc Res*. 1998;38:744–750.
141. Angus JA, Cocks TM, McPherson GA, Broughton A. The acetylcholine paradox: a constrictor of human small coronary arteries even in the presence of endothelium. *Clin Exp Pharmacol Physiol*. 1991;18:33–36.
142. Terata K, Miura H, Liu Y, Loberiza F, Gutterman DD. Human coronary arteriolar dilation to adrenomedullin: role of nitric oxide and K(+) channels. *Am J Physiol Heart Circ Physiol*. 2000;279:H2620–H2626.
143. Sueda S, Fukuda H, Watanabe K, Ochi N, Kawada H, Hayashi Y, Uraoka T. Clinical characteristics and possible mechanism of paroxysmal atrial fibrillation induced by intracoronary injection of acetylcholine. *Am J Cardiol*. 2001;88:570–573.
144. Sato A, Miura H, Liu Y, Somberg LB, Otterson MF, Demeure MJ, Schulte WJ, Eberhardt LM, Loberiza FR, Sakuma I, Gutterman DD. Effect of gender on endothelium-dependent dilation to bradykinin in human adipose microvessels. *Am J Physiol Heart Circ Physiol*. 2002;283:H845–H852. doi: 10.1152/ajpheart.00160.2002.
145. Miura H, Liu Y, Gutterman DD. Human coronary arteriolar dilation to bradykinin depends on membrane hyperpolarization. Contribution of nitric oxide and calcium-activated K+ channels. *Circulation*. 1999;99:3132–3138.
146. Larsen BT, Gutterman DD, Sato A, Toyama K, Campbell WB, Zeldin DC, Manthati VL, Falck JR, Miura H. Hydrogen peroxide inhibits cytochrome p450 epoxygenases: interaction between two endothelium-derived hyperpolarizing factors. *Circ Res*. 2008;102:59–67. doi: 10.1161/CIRCRESAHA.107.159129.
147. Ding Z, Gödecke A, Schrader J. Contribution of cytochrome P450 metabolites to bradykinin-induced vasodilation in endothelial NO synthase deficient mouse hearts. *Br J Pharmacol*. 2002;135:631–638. doi: 10.1038/sj.bjp.0704472.
148. Quayle JM, Nelson MT, Standen NB. ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. *Physiol Rev*. 1997;77:1165–1232.
149. Lynch FM, Austin C, Heagerty AM, Izzard AS. Adenosine- and hypoxia-induced dilation of human coronary resistance arteries: evidence against the involvement of K(ATP) channels. *Br J Pharmacol*. 2006;147:455–458. doi: 10.1038/sj.bjp.0706622.
150. Kemp BK, Cocks TM. Adenosine mediates relaxation of human small resistance-like coronary arteries via A2B receptors. *Br J Pharmacol*. 1999;126:1796–1800. doi: 10.1038/sj.bjp.0702462.
151. Sato A, Terata K, Miura H, Toyama K, Loberiza FR Jr, Hatoum OA, Saito T, Sakuma I, Gutterman DD. Mechanism of vasodilation to adenosine in coronary arterioles from patients with heart disease. *Am J Physiol Heart Circ Physiol*. 2005;288:H1633–H1640. doi: 10.1152/ajpheart.00575.2004.
152. Miura H, Wachtel RE, Loberiza FR Jr, Saito T, Miura M, Nicolosi AC, Gutterman DD. Diabetes mellitus impairs vasodilation to hypoxia in human coronary arterioles: reduced activity of ATP-sensitive potassium channels. *Circ Res*. 2003;92:151–158.
153. Beyer AM, Durand MJ, Hockenberry J, Gamblin TC, Phillips SA, Gutterman DD. An acute rise in intraluminal pressure shifts the mediator of flow-mediated dilation from nitric oxide to hydrogen peroxide in human arterioles. *Am J Physiol Heart Circ Physiol*. 2014;307:H1587–H1593. doi: 10.1152/ajpheart.00557.2014.
154. Matoba T, Shimokawa H, Kubota H, Morikawa K, Fujiki T, Kunihiro I, Mukai Y, Hirakawa Y, Takeshita A. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in human mesenteric arteries. *Biochem Biophys Res Commun*. 2002;290:909–913. doi: 10.1006/bbrc.2001.6278.
155. Morikawa K, Shimokawa H, Matoba T, Kubota H, Akaike T, Talukder MA, Hatanaka M, Fujiki T, Maeda H, Takahashi S, Takeshita A. Pivotal role of Cu,Zn-superoxide dismutase in endothelium-dependent hyperpolarization. *J Clin Invest*. 2003;112:1871–1879. doi: 10.1172/JCI19351.
156. Hatoum OA, Binion DG, Otterson MF, Gutterman DD. Acquired microvascular dysfunction in inflammatory bowel disease: loss of nitric oxide-mediated vasodilation. *Gastroenterology*. 2003;125:58–69.
157. Singh A, Donnino R, Weintraub H, Schwartzbard A. Effect of strict glycemic control in patients with diabetes mellitus on frequency of macrovascular events. *Am J Cardiol*. 2013;112:1033–1038. doi: 10.1016/j.amjcard.2013.05.044.
158. Feng J, Liu Y, Chu LM, Singh AK, Dobrilovic N, Fingleton JG, Clements RT, Bianchi C, Sellke FW. Changes in microvascular reactivity after cardiopulmonary bypass in patients with poorly controlled versus controlled diabetes. *Circulation*. 2012;126:S73–S80. doi: 10.1161/CIRCULATIONAHA.111.084590.
159. Shida T, Nozawa T, Sobajima M, Ihori H, Matsuki A, Inoue H. Fluvastatin-induced reduction of oxidative stress ameliorates diabetic cardiomyopathy in association with improving coronary microvasculature. *Heart Vessels*. 2014;29:532–541. doi: 10.1007/s00380-013-0402-6.

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160. Finck BN, Han X, Courtois M, Aimond F, Nerbonne JM, Kovacs A, Gross RW, Kelly DP. A critical role for PPARalpha-mediated lipotoxicity in the pathogenesis of diabetic cardiomyopathy: modulation by dietary fat content. *Proc Natl Acad Sci U S A*. 2003;100:1226–1231. doi: 10.1073/pnas.0336724100.
161. Dessy C, Moniotte S, Ghisdal P, Havaux X, Noirhomme P, Balligand JL. Endothelial beta3-adrenoceptors mediate vasorelaxation of human coronary microarteries through nitric oxide and endothelium-dependent hyperpolarization. *Circulation*. 2004;110:948–954. doi: 10.1161/01.CIR.0000139331.85766.AF.
161a. Zinkevich NS, Wittenburg AL, Gutterman DD. Role of cyclooxygenase in flow-induced dilation of human coronary arterioles depends upon age. *Circulation*. 2010;122:A15764. Abstract. http://circ.ahajournals.org/cgi/content/meeting_abstract/122/21_MeetingAbstracts/A15764.
162. Kichuk MR, Seyedi N, Zhang X, Marboe CC, Michler RE, Addonizio LJ, Kaley G, Nasjletti A, Hintze TH. Regulation of nitric oxide production in human coronary microvessels and the contribution of local kinin formation. *Circulation*. 1996;94:44–51.
163. Rubin MA, Putzi M, Mucci N, Smith DC, Wojno K, Korenchuk S, Pienta KJ. Rapid ("warm") autopsy study for procurement of metastatic prostate cancer. *Clin Cancer Res*. 2000;6:1038–1045.
164. Nelson MT, Quayle JM. Physiological roles and properties of potassium channels in arterial smooth muscle. *Am J Physiol*. 1995;268:C799–C822.
165. Ko EA, Han J, Jung ID, Park WS. Physiological roles of K+ channels in vascular smooth muscle cells. *J Smooth Muscle Res*. 2008;44:65–81.
166. Wilde DW, Lee KS. Outward potassium currents in freshly isolated smooth muscle cell of dog coronary arteries. *Circ Res*. 1989;65:1718–1734.
167. Aiello EA, Clement-Chomienne O, Sontag DP, Walsh MP, Cole WC. Protein kinase C inhibits delayed rectifier K+ current in rabbit vascular smooth muscle cells. *Am J Physiol*. 1996;271:H109–H119.
168. Rogers PA, Dick GM, Knudson JD, Focardi M, Bratz IN, Swafford AN Jr, Saitoh S, Tune JD, Chilian WM. H2O2-induced redox-sensitive coronary vasodilation is mediated by 4-aminopyridine-sensitive K+ channels. *Am J Physiol Heart Circ Physiol*. 2006;291:H2473–H2482. doi: 10.1152/ajpheart.00172.2006.
169. Brayden JE, Nelson MT. Regulation of arterial tone by activation of calcium-dependent potassium channels. *Science*. 1992;256:532–535.
170. Archer SL, Gragasin FS, Wu X, Wang S, McMurtry S, Kim DH, Platonov M, Koshal A, Hashimoto K, Campbell WB, Falck JR, Michelakis ED. Endothelium-derived hyperpolarizing factor in human internal mammary artery is 11,12-epoxyeicosatrienoic acid and causes relaxation by activating smooth muscle BK(Ca) channels. *Circulation*. 2003;107:769–776.
171. Fürstenau M, Löhn M, Ried C, Luft FC, Haller H, Gollasch M. Calcium sparks in human coronary artery smooth muscle cells resolved by confocal imaging. *J Hypertens*. 2000;18:1215–1222.
172. Li Z, Lu N, Shi L. Exercise training reverses alterations in Kv and BKCa channel molecular expression in thoracic aorta smooth muscle cells from spontaneously hypertensive rats. *J Vasc Res*. 2014;51:447–457. doi: 10.1159/000369928.
173. Jackson WF. Potassium channels in the peripheral microcirculation. *Microcirculation*. 2005;12:113–127. doi: 10.1080/10739680590896072.
174. Stephens GJ, Owen DG, Robertson B. Cysteine-modifying reagents alter the gating of the rat cloned potassium channel Kv1.4. *Pflugers Arch*. 1996;431:435–442.
175. Duprat F, Guillemare E, Romey G, Fink M, Lesage F, Lazdunski M, Honore E. Susceptibility of cloned K+ channels to reactive oxygen species. *Proc Natl Acad Sci U S A*. 1995;92:11796–11800.
176. Li X, Tang K, Xie B, Li S, Rozanski GJ. Regulation of Kv4 channel expression in failing rat heart by the thioredoxin system. *Am J Physiol Heart Circ Physiol*. 2008;295:H416–H424. doi: 10.1152/ajpheart.91446.2007.
177. Mittal M, Gu XQ, Pak O, Pamenter ME, Haag D, Fuchs DB, Schermuly RT, Ghofrani HA, Brandes RP, Seeger W, Grimminger F, Haddad GG, Weissmann N. Hypoxia induces Kv channel current inhibition by increased NADPH oxidase-derived reactive oxygen species. *Free Radic Biol Med*. 2012;52:1033–1042. doi: 10.1016/j.freeradbiomed.2011.12.004.
178. Jerng HH, Pfaffinger PJ. S-glutathionylation of an auxiliary subunit confers redox sensitivity to Kv4 channel inactivation. *PLoS One*. 2014;9:e93315. doi: 10.1371/journal.pone.0093315.
179. Li H, Chai Q, Gutterman DD, Liu Y. Elevated glucose impairs cAMP-mediated dilation by reducing Kv channel activity in rat small coronary smooth muscle cells. *Am J Physiol Heart Circ Physiol*. 2003;285:H1213–H1219. doi: 10.1152/ajpheart.00226.2003.
180. Liu Y, Gutterman DD. The coronary circulation in diabetes: influence of reactive oxygen species on K+ channel-mediated vasodilation. *Vascul Pharmacol*. 2002;38:43–49.
181. Liu Y, Terata K, Rusch NJ, Gutterman DD. High glucose impairs voltage-gated K(+) channel current in rat small coronary arteries. *Circ Res*. 2001;89:146–152.
182. Li H, Gutterman DD, Rusch NJ, Bubolz A, Liu Y. Nitration and functional loss of voltage-gated K+ channels in rat coronary microvessels exposed to high glucose. *Diabetes*. 2004;53:2436–2442.
183. Heaps CL, Tharp DL, Bowles DK. Hypercholesterolemia abolishes voltage-dependent K+ channel contribution to adenosine-mediated relaxation in porcine coronary arterioles. *Am J Physiol Heart Circ Physiol*. 2005;288:H568–H576. doi: 10.1152/ajpheart.00157.2004.
184. Heaps CL, Jeffery EC, Laine GA, Price EM, Bowles DK. Effects of exercise training and hypercholesterolemia on adenosine activation of voltage-dependent K+ channels in coronary arterioles. *J Appl Physiol (1985)*. 2008;105:1761–1771. doi: 10.1152/japplphysiol.90958.2008.
185. Caouette D, Dongmo C, Bérubé J, Fournier D, Daleau P. Hydrogen peroxide modulates the Kv1.5 channel expressed in a mammalian cell line. *Naunyn Schmiedebergs Arch Pharmacol*. 2003;368:479–486. doi: 10.1007/s00210-003-0834-0.
186. Platoshyn O, Yu Y, Golovina VA, McDaniel SS, Krick S, Li L, Wang JY, Rubin LJ, Yuan JX. Chronic hypoxia decreases K(V) channel expression and function in pulmonary artery myocytes. *Am J Physiol Lung Cell Mol Physiol*. 2001;280:L801–L812.
187. Wang J, Weigand L, Wang W, Sylvester JT, Shimoda LA. Chronic hypoxia inhibits Kv channel gene expression in rat distal pulmonary artery. *Am J Physiol Lung Cell Mol Physiol*. 2005;288:L1049–L1058. doi: 10.1152/ajplung.00379.2004.
188. Fountain SJ, Cheong A, Li J, Dondas NY, Zeng F, Wood IC, Beech DJ. K(v)1.5 potassium channel gene regulation by Sp1 transcription factor and oxidative stress. *Am J Physiol Heart Circ Physiol*. 2007;293:H2719–H2725. doi: 10.1152/ajpheart.00637.2007.
189. Park SW, Noh HJ, Sung DJ, Kim JG, Kim JM, Ryu SY, Kang K, Kim B, Bae YM, Cho H. Hydrogen peroxide induces vasorelaxation by enhancing 4-aminopyridine-sensitive Kv currents through S-glutathionylation. *Pflugers Arch*. 2014;467:285–297. doi: 10.1007/s00424-014-1513-3.
190. Cogolludo A, Frazziano G, Cobeño L, Moreno L, Lodi F, Villamor E, Tamargo J, Perez-Vizcaino F. Role of reactive oxygen species in Kv channel inhibition and vasoconstriction induced by TP receptor activation in rat pulmonary arteries. *Ann N Y Acad Sci*. 2006;1091:41–51. doi: 10.1196/annals.1378.053.
191. Archer SL, Gomberg-Maitland M, Maitland ML, Rich S, Garcia JG, Weir EK. Mitochondrial metabolism, redox signaling, and fusion: a mitochondria-ROS-HIF-1alpha-Kv1.5 O2-sensing pathway at the intersection of pulmonary hypertension and cancer. *Am J Physiol Heart Circ Physiol*. 2008;294:H570–H578. doi: 10.1152/ajpheart.01324.2007.
192. Svoboda LK, Reddie KG, Zhang L, Vesely ED, Williams ES, Schumacher SM, O'Connell RP, Shaw R, Day SM, Anumonwo JM, Carroll KS, Martens JR. Redox-sensitive sulfenic acid modification regulates surface expression of the cardiovascular voltage-gated potassium channel Kv1.5. *Circ Res*. 2012;111:842–853. doi: 10.1161/CIRCRESAHA.111.263525.
193. Adda S, Fleischmann BK, Freedman BD, Yu M, Hay DW, Kotlikoff MI. Expression and function of voltage-dependent potassium channel genes in human airway smooth muscle. *J Biol Chem*. 1996;271:13239–13243.
194. Brundel BJ, Van Gelder IC, Henning RH, Tieleman RG, Tuinenburg AE, Wietses M, Grandjean JG, Van Gilst WH, Crijns HJ. Ion channel remodeling is related to intraoperative atrial effective refractory periods in patients with paroxysmal and persistent atrial fibrillation. *Circulation*. 2001;103:684–690.
195. Van Wagoner DR, Pond AL, McCarthy PM, Trimmer JS, Nerbonne JM. Outward K+ current densities and Kv1.5 expression are reduced in chronic human atrial fibrillation. *Circ Res*. 1997;80:772–781.
196. Ohanyan V, Yin L, Bardakjian R, et al. Requisite Role of Kv1.5 Channels in Coronary Metabolic Dilation. *Circ Res*. 2015;117:612–621. doi: 10.1161/CIRCRESAHA.115.306642.
197. Nelson MT, Quayle JM. Physiological roles and properties of potassium channels in arterial smooth muscle. *Am J Physiol*. 1995;268:C799–C822.
198. Carl A, Lee HK, Sanders KM. Regulation of ion channels in smooth muscles by calcium. *Am J Physiol*. 1996;271:C9–34.

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199. Jackson WF, Huebner JM, Rusch NJ. Enzymatic isolation and characterization of single vascular smooth muscle cells from cremasteric arterioles. *Microcirculation*. 1997;4:35–50.
200. Jackson WF, Blair KL. Characterization and function of Ca(2+)-activated K+ channels in arteriolar muscle cells. *Am J Physiol*. 1998;274:H27–H34.
201. Li PL, Jin MW, Campbell WB. Effect of selective inhibition of soluble guanylyl cyclase on the K(Ca) channel activity in coronary artery smooth muscle. *Hypertension*. 1998;31:303–308.
202. Zhu S, Han G, White RE. PGE2 action in human coronary artery smooth muscle: role of potassium channels and signaling cross-talk. *J Vasc Res*. 2002;39:477–488.
203. White RE, Kryman JP, El-Mowafy AM, Han G, Carrier GO. cAMP-dependent vasodilators cross-activate the cGMP-dependent protein kinase to stimulate BK(Ca) channel activity in coronary artery smooth muscle cells. *Circ Res*. 2000;86:897–905.
204. Campbell WB, Gebremedhin D, Pratt PF, Harder DR. Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. *Circ Res*. 1996;78:415–423.
205. Xi Q, Cheranov SY, Jaggar JH. Mitochondria-derived reactive oxygen species dilate cerebral arteries by activating Ca2+ sparks. *Circ Res*. 2005;97:354–362. doi: 10.1161/01.RES.0000177669.29525.78.
206. Asano M, Matsuda T, Hayakawa M, Ito KM, Ito K. Increased resting Ca2+ maintains the myogenic tone and activates K+ channels in arteries from young spontaneously hypertensive rats. *Eur J Pharmacol*. 1993;247:295–304.
207. Liu Y, Pleyte K, Knaus HG, Rusch NJ. Increased expression of Ca2+-sensitive K+ channels in aorta of hypertensive rats. *Hypertension*. 1997;30:1403–1409.
208. Liu Y, Hudetz AG, Knaus HG, Rusch NJ. Increased expression of Ca2+-sensitive K+ channels in the cerebral microcirculation of genetically hypertensive rats: evidence for their protection against cerebral vasospasm. *CircRes*. 1998;82:729–737.
209. Wiecha J, Schläger B, Voisard R, Hannekum A, Mattfeldt T, Hombach V. Ca(2+)-activated K+ channels in human smooth muscle cells of coronary atherosclerotic plaques and coronary media segments. *Basic Res Cardiol*. 1997;92:233–239.
210. Lu T, He T, Katusic ZS, Lee HC. Molecular mechanisms mediating inhibition of human large conductance Ca2+-activated K+ channels by high glucose. *Circ Res*. 2006;99:607–616. doi: 10.1161/01.RES.0000243147.41792.93.
211. Tang XD, Garcia ML, Heinemann SH, Hoshi T. Reactive oxygen species impair Slo1 BK channel function by altering cysteine-mediated calcium sensing. *Nat Struct Mol Biol*. 2004;11:171–178. doi: 10.1038/nsmb725.
212. Liu Y, Terata K, Chai Q, Li H, Kleinman LH, Gutterman DD. Peroxynitrite inhibits Ca2+-activated K+ channel activity in smooth muscle of human coronary arterioles. *Circ Res*. 2002;91:1070–1076.
213. Miller AW, Dimitropoulou C, Han G, White RE, Busija DW, Carrier GO. Epoxyeicosatrienoic acid-induced relaxation is impaired in insulin resistance. *Am J Physiol Heart Circ Physiol*. 2001;281:H1524–H1531.
214. Prieto D, Rivera L, Benedito S, Recio P, Villalba N, Hernández M, García-Sacristán A. Ca2+-activated K+ (KCa) channels are involved in the relaxations elicited by sildenafil in penile resistance arteries. *Eur J Pharmacol*. 2006;531:232–237. doi: 10.1016/j.ejphar.2005.12.033.
215. Nishijima Y, Zhang DX. H2O2-induced dilation in human adipose arterioles: role of smooth muscle K+ channels. *FASEB J*. 2015;29:637.4.

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