STATE-OF-THE-ART PAPERS

Endothelial Dysfunction, Arterial Stiffness, and Heart Failure

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Outcomes for heart failure (HF) patients remain suboptimal. No known therapy improves mortality in acute HF and HF with preserved ejection fraction; the most recent HF trial results have been negative or neutral. Improvement in surrogate markers has not necessarily translated into better outcomes. To translate breakthroughs with potential therapies into clinical benefit, a better understanding of the pathophysiology establishing the foundation of benefit is necessary. Vascular function plays a central role in the development and progression of HF. Endothelial function and nitric oxide availability affect myocardial function, systemic and pulmonary hemodynamics, and coronary and renal circulation. Arterial stiffness modulates ventricular loading conditions and diastolic function, key components of HF with preserved ejection. Endothelial function and arterial stiffness may therefore serve as important physiological targets for new HF therapies and facilitate patient selection for improved application of existing agents. (J Am Coll Cardiol 2012;60:1455–69) © 2012 by the American College of Cardiology Foundation

Need for Novel Therapeutic Targets for Heart Failure

The public health impact and the need to intervene on the growing heart failure (HF) epidemic are in the center of the national healthcare debate. HF is the primary cause of >1 million hospitalizations annually and is associated with a postdischarge mortality and readmission rate of approximately 45% at 60 to 90 days (1,2). With the population aging, the already alarming HF epidemic is projected to worsen. Despite advances in drug and device therapy for chronic HF with reduced ejection fraction (EF), outcomes at the community level remain suboptimal (3,4). Although many therapies have been evaluated within the last decade, few have produced positive results in Phase III trials (5–13). Notably, improvement in surrogate markers in Phase II studies has not necessarily translated into better clinical outcomes (14). For example, improved hemodynamics with nesiritide (15) and promising renoprotective effects of rolofylline did not result in reduced mortality or hospitalization rates (16,17). Selective V2 receptor vasopressin antagonists likewise failed to improve outcomes despite showing promise in initial studies, with the effects of unopposed V1 receptor activity not being fully realized (6,7). Furthermore, targeting many of the consequences of altered physiology

linked to HF outcomes (e.g., ischemia [18,19], hyperuricemia [20], renal dysfunction [17], hyponatremia [6,7], ventricular arrhythmias [21]) has not translated consistently into improved clinical outcomes either. There are, however, other examples in which an approach of targeting a biologic surrogate did improve clinical outcomes; for example, defibrillator therapy for prevention of sudden cardiac death (22).

Considering the persistent suboptimal outcomes for chronic HF with reduced EF, the lack of an agent that improves survival for HF with preserved EF or acute HF, and the many recent negative or neutral HF trials, newer therapeutic targets warrant consideration (23–25). Successful translation of breakthroughs to meaningful clinical benefit requires a deeper understanding of the relevant pathophysiology. Mechanistic pilot studies using surrogate markers that establish a solid foundation of therapeutic benefit may bridge this missing translational step and allow for more comprehensive and relevant evaluation of therapeutic agents before resource-intensive Phase III trials. We propose that for a novel agent or therapeutic target to be considered for Phase II and III clinical trials, it should fulfill the requirements illustrated in Figure 1.

Recently, novel mechanistic pathways of endothelial dysfunction and arterial stiffness in HF have been investigated. These may provide the rationale for new drug development and allow for improved application of existing agents. We therefore discuss the role of vascular function measures as potential targets for new HF therapeutic development and research.

Literature Search and Selection Strategy

A search of Medline, PubMed, EMBASE, and Evidence Based Medicine Reviews database including Cochrane Database of Systematic Reviews, ACP Journal Club, Database of Abstracts of Reviews of Effects, Cochrane Central Register of Controlled Trials, Health Technology Assessment, and Cochrane Methodology Register was performed to identify all studies that evaluated the effects of endothelial function and arterial stiffness in HF published up to April 1, 2011, without any language or publication form restriction. The keywords of "heart failure," "cardiomyopathy," "systolic function," "systolic dysfunction," "diastolic dysfunction," "human," and "endothelial" or "arterial stiffness" were used to conduct the literature search, which identified >4,000 publications. Subsequently, studies other than those in the English language were excluded. In addition, publications without original data (reviews, letters, and editorials) or with a primary focus on non-HF issues (e.g., coronary artery disease) were excluded as well. References for these studies were cross-checked to obtain additional studies that may have been missed by the original search. Finally, key papers from this search that highlighted the important concepts presented in this review were selected.

Endothelial Function as Potential Target

Normal endothelial function. Endothelium is a monolayer of cells covering the inner surface of blood vessels, and it acts as a functional and structural barrier between blood and the vessel wall, preventing platelet and leukocyte adhesion and aggregation, controlling permeability to plasma components, and modulating blood flow (Fig. 2). The healthy endothelium is a dynamic organ that regulates vascular tone by balancing production of vasodilators and vasoconstrictors in response to a variety of stimuli (26). Nitric oxide (NO), the predominant mediator of normal vascular function, is released by the endothelium and diffuses within the vessel wall, causing smooth muscle dilation and myofibrillar relaxation in response to stimulation by endogenous factors such as bradykinin, acetylcholine, and catecholamines, as well as ischemia, temperature change, and mechanical stimuli, including shear stress (27). Endothelium also provides antiproliferative and anti-inflammatory actions, and regulates fibrinolysis as well as the coagulation pathway through the balanced production of anticoagulant (e.g., tissue plasminogen activator, thrombomodulin) and procoagulant (e.g., tissue factor, von Willebrand factor) factors, which maintain hemostatic properties of blood vessels (28).

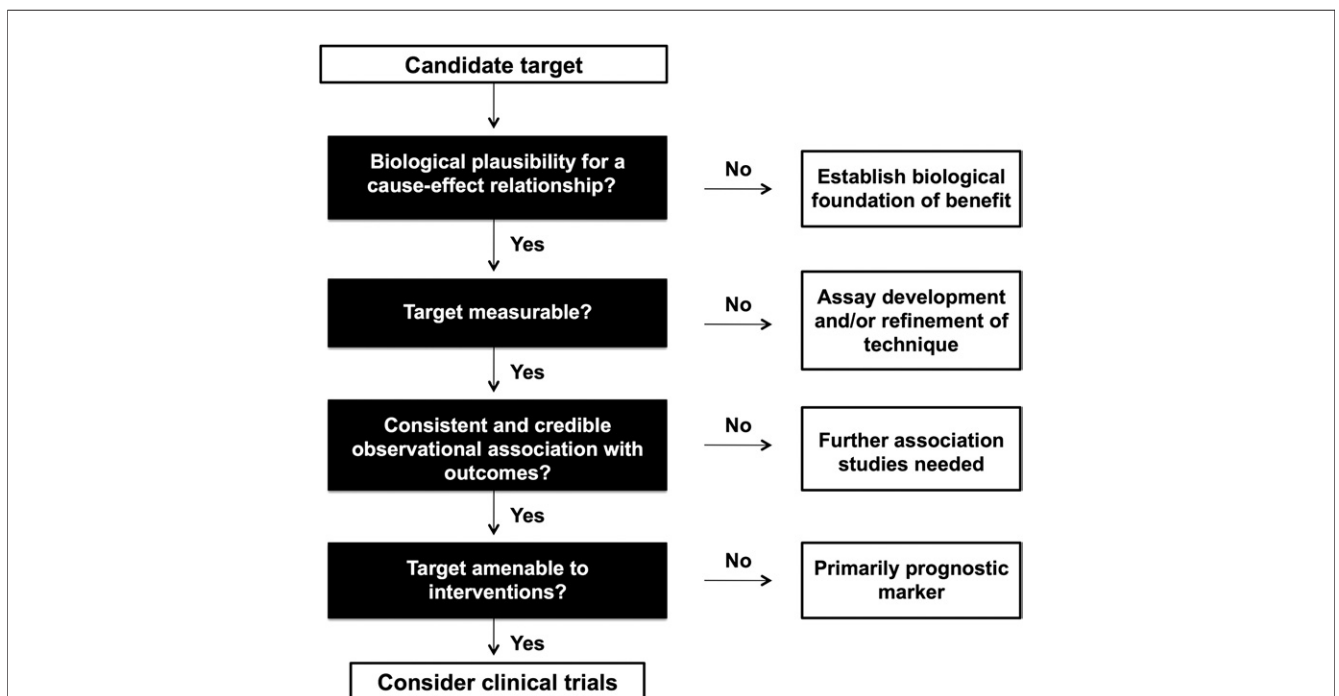**Central role of NO.** NO is synthesized from L-arginine by NO synthase (NOS) (29). The 3 main NOS isoforms include

Figure 1   **Proposed Schema for Evaluation of Candidate Targets for Clinical Trials**
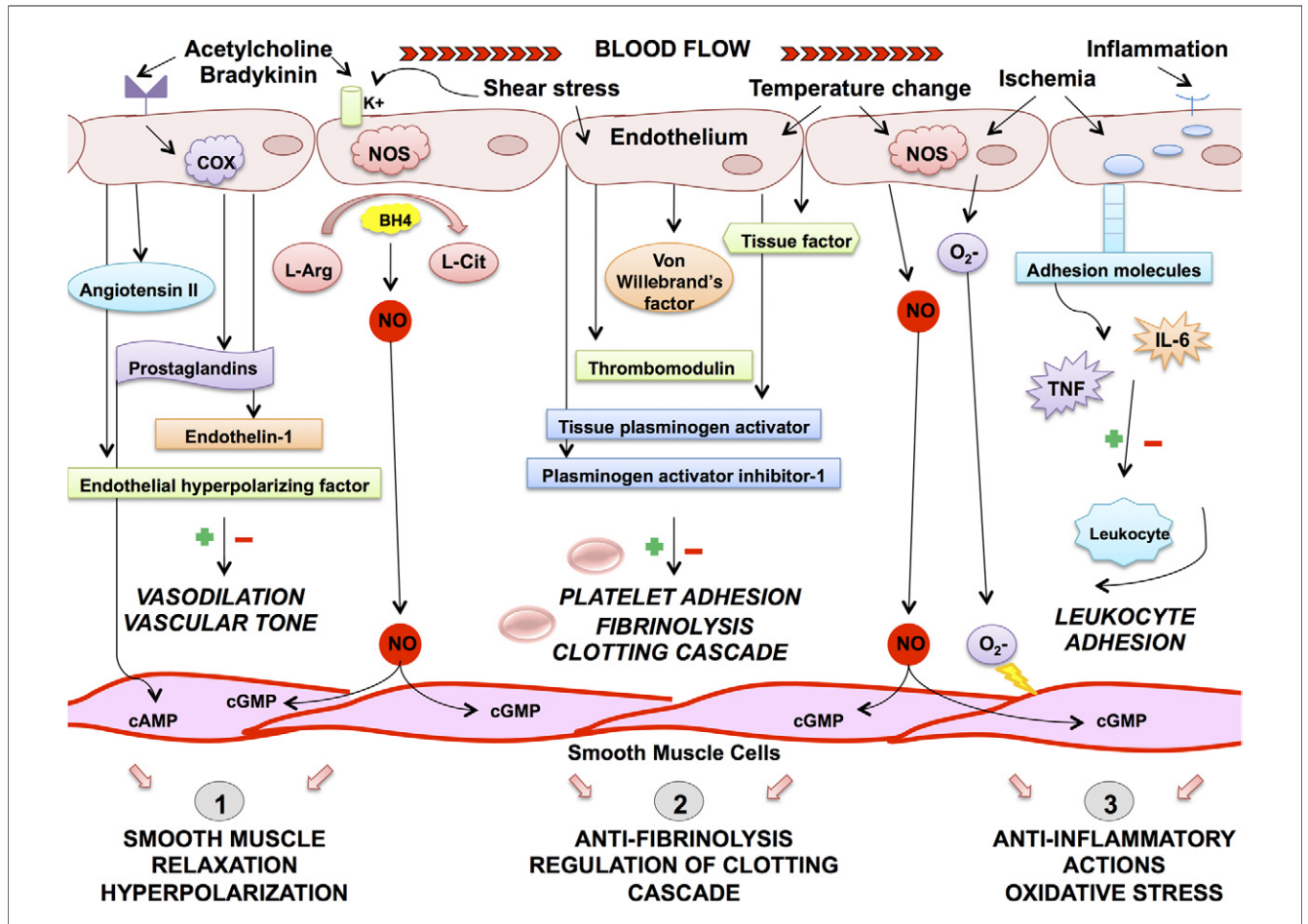
Figure 2   **Normal Endothelial Function**

The endothelium is responsible for a number of physiological functions, including: 1) regulation of vascular tone through balanced production of vasodilators and vaso-constrictors; 2) control of blood fluidity and coagulation through production of factors that regulate platelet activity, the clotting cascade, and the fibrinolytic system; and 3) regulation of inflammatory processes through expression of cytokines and adhesion molecules. cAMP = cyclic adenosine monophosphate; cGMP = cyclic guanosine monophosphate; COX = cyclooxygenase; BH4 = tetrahydrobiopterin; IL = interleukin; TNF = tumor necrosis factor; L-arg = L-arginine; L-cit = L-citrulline; NO = nitric oxide; NOS = nitric oxide synthase; O_2- = superoxide.

constitutive endothelial NOS (eNOS or NOS3), neuronal NOS (or NOS1), and inducible NOS (iNOS) that are differently coexpressed in NO-producing cells and also inducible by immunological stimuli (30). Although NO produced by all 3 pathways regulates normal physiology, large amounts of NO produced by iNOS may have a cytotoxic effect and inhibit myocardial contractility (31). Because HF triggers changes in myocardial NO production, shifting from spatially and temporally regulated NO production by eNOS to excessive release by iNOS, the distinction between NO produced by eNOS/neuronal NOS or iNOS is important (32,33). In the intact endothelium, hormonal and physical stimuli cause the constitutively expressed eNOS to generate NO, which then diffuses into smooth muscle cells and stimulates soluble guanylate cyclase (sGC) to produce cyclic guanine monophosphate, which causes smooth muscle relaxation and also has antiprolifera-tive effects. In addition to these smooth muscle cell–mediated vascular effects, NO targets neighboring extravas-

cular tissues, including myocardium (34). Release of endothelial progenitor cells from bone marrow, which has been shown to repair damaged endothelium, is also partially NO dependent (35). Furthermore, NO can act as an endocrine vasoregulator, modulating blood flow in the microcirculation when vehiculated by S-nitrosohemoglobin, which transports and releases NO to areas of tissue hypoxia or increased oxygen extraction (36). Importantly, disruption of NO delivery to the microcirculation contributes to vasoconstriction and uncoupling of oxygen delivery in skeletal muscle. Given the pivotal role of NO in mediating endothelial function, impairment of vasodilation due to decreased NO availability is often used as a measure of endothelial function (37,38).

Endothelial dysfunction in HF. Although endothelial dysfunction has traditionally been associated with systemic vasoconstriction in advanced HF, newer insights suggest a more central role in HF pathogenesis (39–45). The failing heart is characterized by an altered redox state with over-

production of reactive oxygen species, and there is increasing evidence to suggest that the abnormal cardiac and vascular phenotypes characterizing the failing heart are caused in large part by imbalances between NO bioavailability and oxidative stress (46). In HF, neurohumoral activation, release of inflammatory messengers from the myocardium, and altered local shear forces modulate gene expression and promote atherogenesis, increasing oxidative stress and reducing production of NO (47,48). The resulting endothelial dysfunction triggers an increase in the production of cytokines, down-regulation or uncoupling of eNOS (32,33), and further increases in oxidative stress (49,50). These processes culminate in reduced NO bioavailability and worsening endothelial dysfunction, which in turn propagates development and progression of HF (41,42,51,52). These abnormalities have emerged as a common pathophysiological element in the development and progression of HF and are also associated with HF risk factors (53). Within this construct, myocardial adverse effects and endothelial dysfunction related to oxidative stress represent a unifying feature that drives both the symptoms and unfavorable outcomes associated with both ischemic and nonischemic HF (54).

Chronic HF. Increasing HF severity is associated with NO imbalance and endothelial dysfunction that manifests in different forms (52,53). Besides increasing afterload due to systemic (55) and pulmonary vascular constriction (56,57), altered endothelial function underlies regional vasomotor dysregulation in the renal (58) and coronary circulation (59). Decreased coronary endothelium-dependent vasodilator capacity impairs myocardial perfusion, reduces coronary flow (60,61), and worsens ventricular function (53). The dysfunctional endothelium contributes to increased vascular stiffness and impaired arterial distensibility, augmenting myocardial damage (62–64). NO imbalances also alter matrix metalloproteinases, which affect cell migration, cardiac hypertrophy, and atherosclerotic plaque stability (65). Increased endothelin-1 in HF causes increased vascular resistance, smooth muscle cell growth, and matrix production, resulting in vascular remodeling, endothelial dysfunction, and HF progression. Reduced NO in HF affects endothelial progenitor cells, disabling endothelial repair and regeneration (35). Circulating cytokines, particularly tumor necrosis factor-alpha, down-regulate eNOS expression (32,66) and are related to the degree of endothelial dysfunction in HF (67), which also correlates with progressive deterioration in functional class (68). Furthermore, serum from patients with HF has been shown to induce endothelial cell apoptosis (32) through eNOS down-regulation (69); recently, a common polymorphism of eNOS (Asp298), linked with decreased NOS activity, was associated with poorer survival in HF (70). However, promising research demonstrates that targeted overexpression of eNOS may attenuate both cardiac and pulmonary dysfunction (71). Importantly, severity of endothelial dysfunction is also related to exercise capacity (54,72). In HF, reduced blood flow and sheer stress results in impaired exercise-induced

NO release, affecting muscle function (73–75), exercise capacity, and ventilation (76–78). Down-regulation of eNOS shifts catabolism from free fatty acids to lactate, worsening exercise tolerance. Endothelial dysfunction also affects autonomic balance, decreasing vagal and increasing adrenergic activity, thus further worsening chronic HF (79).

Acute HF. NO-dependent regulation of ventricular function and vascular tone also determines hemodynamic status in acute HF. Decreased NO availability induces vasoconstriction and increased vascular stiffness in the systemic and pulmonary circulation, resulting in augmented left ventricular (LV) and right ventricular systolic workload. Decreased NO bioavailability also enhances endothelin-1–induced vasoconstriction (80), increases sympathetic outflow and catecholamine release (81), and diminishes sodium excretion in the kidney (82), all of which are important in the vicious circle of acute HF syndrome. Excess reactive oxygen species react with NO, disrupting physiological signaling and leading to production of toxic and reactive molecules, notably peroxynitrite (83). Oxidative stress, quantifiable clinically through urine isoprostane levels and plasma aminothiols (84,85), is increased in acute decompensated HF (86), thus unfavorably shifting the nitroso–redox balance and the ventricular and vascular effects of NO.

Renal dysfunction. NO imbalance drives vasomotor nephropathy, which underlies acute renal damage and the cardiorenal syndrome in HF (58,87). This action is in part due to reduced renal flow from inappropriate arteriolar vasoconstriction superimposed on baseline low cardiac output. Intrarenal NO regulates glomerular hemodynamics (88), tubular transport, and tubuloglomerular feedback. NO relaxes both afferent and efferent arterioles and regulates renal medullary blood flow as well. In the proximal tubule, NO promotes fluid and HCO_3^- reabsorption and inhibits Na^+/H^+ exchanger (89) and Na^+-K^+ adenosine trophosphatase activity. In the ascending loop of Henle, NO inhibits Cl^- and HCO_3^- reabsorption (90–92) and in the collecting duct it decreases Na^+ and fluid reabsorption (93–95). The net result is increased renal and glomerular perfusion, natriuresis, and diuresis (96,97). Thus, NO imbalance affects renal function, worsening HF.

Pulmonary hypertension and right ventricular failure. In the pulmonary vasculature, dysfunctional endothelium can affect vascular tone (98–100). Secondary pulmonary hypertension and right ventricular dysfunction is common in HF (101–104) and affect prognosis (105–108). Elevated pulmonary vascular resistance in HF results from smooth muscle tone dysregulation and remodeling of the pulmonary vasculature (57). These abnormalities are in part attributed to pulmonary vascular endothelial dysfunction, resulting from impaired NO availability and increased endothelin-1 expression (57). In HF, NO-dependent pulmonary vasodilation is impaired (109–111), suggesting a potential therapeutic role for agents that improve endothelial function on pulmonary hypertension and right ventricular function.

Endothelial Function Assessment

Invasive assessment. Vasodilation in response to specific endothelium-dependent and -independent stimuli within the forearm, coronary, or peripheral circulations can be measured to assess endothelial function. Coronary endothelial function can be evaluated by intracoronary infusion of endothelium-dependent vasodilators (e.g., acetylcholine) (112). Changes in conduit vessel diameter measured with quantitative angiography and blood flow with intracoronary Doppler wire are used as measures of conductance and resistance vessel endothelial function, respectively (113,114). Normal response is dilation of epicardial vessels and microcirculation. In endothelial dysfunction, epicardial dilation is attenuated or paradoxical constriction occurs, secondary to the direct smooth muscle constricting effects of acetylcholine, which overrides the dilating effects of endothelium-dependent NO release (115). In other vascular beds, a diminished dilator response is observed, but constriction is rare (116). Endothelium-independent function is assessed by measuring dose response to increasing concentrations of vasodilators that donate NO directly (e.g., nitroglycerin, nitroprusside). Adenosine causes vasodilation by stimulating receptors in the microcirculation, facilitating measurement of the endothelium-independent flow reserve in the microcirculation. Noninvasive evaluation of coronary microvascular function by echocardiography, magnetic resonance imaging, and positron emission tomography is evolving (117–121).

Venous occlusion plethysmography. Venous occlusion plethysmography is used to study forearm blood flow (122) and involves arresting venous outflow with an inflated cuff around the arm enough to occlude venous outflow while preserving arterial inflow (approximately 40 mm Hg) and simultaneously excluding the hand from the circulation by inflating a wrist cuff to suprasystolic pressures (approximately 200 mm Hg). The rate and degree of swelling reflect forearm vascular resistance, whereas the volume, measured by using a voltage-dependent strain gauge, increases in direct proportion to forearm blood flow. A minimally invasive, modified strain-gauge method may be applied to investigate in vivo endothelial function (123). This technique allows manipulation of vascular resistance by administering endothelial agonists (e.g., acetylcholine) and direct smooth muscle relaxants (e.g., nitrates) locally without systemic effects. Simultaneous contralateral arm measurements are used to verify the absence of systemic effects of drug infusion. Venous occlusion plethysmography is usually well tolerated and is highly reproducible (124).

Flow-mediated dilation. With this technique, change in brachial artery diameter is measured by using high-resolution ultrasound (125). After a straight, nonbranching segment of the artery above the antecubital fossa is imaged, a blood pressure cuff placed below the antecubital fossa is inflated to suprasystolic pressure (126). After cuff release, reactive hyperemia is quantified (Fig. 3A) (127). Using electrocardiographic gating, the arterial diameter is recorded at end diastole to determine the response to flow increase, and changes in the arterial diameter are assessed by using digital edge detection (38). Flow-mediated dilation (FMD) is expressed as percent change in diameter from baseline. Response to the endothelium-independent dilator (e.g., nitroglycerin) is also assessed. FMD correlates with coronary endothelial function (128). Aging, body mass index, blood pressure, and smoking lower FMD, and beneficial lifestyle changes such as exercise training and medical therapy (e.g., statins) improve FMD (129,130). This technique, however, is operator dependent (131–133).

Peripheral arterial tonometry. Fingertip peripheral artery tonometry (PAT) is a noninvasive technique that consists of probes with inflatable latex air cuffs connected by pneumatic tubes to an inflating device (134). A constant counterpressure, determined by using baseline diastolic blood pressure, is applied through air cushions preventing venous pooling, thereby avoiding veno-arteriolar reflex vasoconstriction. Pulsatile volume changes in the distal digit induce pressure alterations in the cuff, which are sensed by transducers. A decrease in the arterial blood volume causes a decrease in arterial column changes and is reflected as a decreased PAT signal, and vice versa (Fig. 3B). Endothelial function is measured via a reactive hyperemia PAT index. A computer algorithm calculates the ratio of reactive hyperemic response to basal flow, indexed to the contralateral control arm. PAT hyperemic flow is believed to depend on NO (135), and the ratio correlates with coronary endothelial function (136), FMD (137), and myocardial perfusion imaging studies (134). The possible incremental value of PAT was demonstrated in the Framingham cohort as well (138). However, results from Framingham have also raised questions about its specificity for NO, as PAT was not associated with hypertension, diabetes, or increased age, all of which have been linked to large-artery endothelial dysfunction (139,140). More data are needed to establish the role of PAT.

Endothelial Dysfunction and HF Outcomes

Endothelial dysfunction is related to HF initiation and progression (141) and is associated with adverse outcomes in those with symptomatic and asymptomatic LV dysfunction (59,142,143) and in acute and chronic HF (44,144–147). The degree of endothelial dysfunction correlates with HF severity and functional capacity (54,148). Endothelial dysfunction independently predicts major clinical events in HF (147), including mortality risk (141,146,149,150). In patients with and without coronary artery disease, presence of epicardial or microvascular endothelial dysfunction predicts death (151–156). Endothelial dysfunction is also associated with HF risk factors (e.g., hypertension, diabetes) (152,157). Preservation of endothelial function in HF is associated with improved LV function (144), and recovery is related to improved outcomes (158). In HF, impaired FMD of the brachial artery is common and is associated with poor outcomes irrespective of etiology (54,149,150). Abnormal
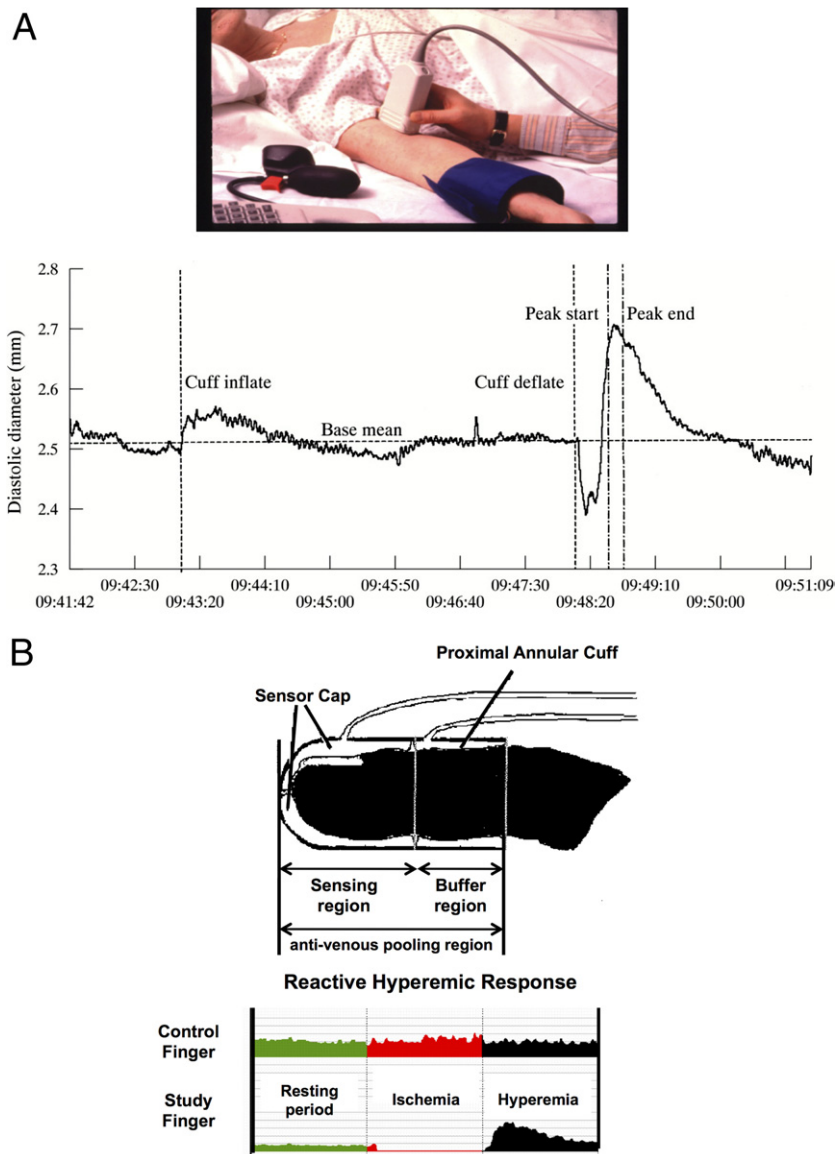
Figure 3   Endothelial Function Measurement Techniques

(A) Flow-mediated dilation. Graph of brachial artery diameter versus time in a normal subject. Variation in measured diameter caused by respiration is visible as a low-amplitude oscillation. Reprinted, with permission, from Sidhu et al. (127). **(B)** Peripheral arterial tonometry. The sensing region is thimble shaped and imparts a 2-point clamping effect to hold it in place while measuring pulsatile volume changes. The adjacent annular cuff provides a buffering effect.

FMD predicts incident cardiovascular events in older adults, a population that has a lower FMD and is also often at increased HF risk (159). Impaired brachial artery FMD identifies patients who will respond to cardiac resynchronization therapy (72), and FMD in addition to B-type natriuretic peptide provides incremental prognostic information in HF (54). The interobserver and intraobserver variability and changes in FMD over time have enabled construction of power curves for clinical trial protocols (160), facilitating the use of FMD in trials.

Race and sex-related differences. Although women have higher FMD and PAT ratios, they also have a higher preva-

lence of abnormal brachial and digital vascular function (161). Racial differences in distribution of blood flow at rest and during stress may also be due to differences in endothelial function. Black patients with HF have lower resting flow, exercise-induced vasodilation, and hyperemic blood flow (162). Furthermore, both conduit and resistance vessel endothelial function are significantly decreased in black patients, which correlates with reduced NO-dependent vasodilation during stress (163). The reduced NO activity in black patients is partly due to enhanced NO inactivation by oxidative stress (164) and may contribute to the observed racial differences with vasodilator therapy for HF (165).

Arterial Stiffness as a Therapeutic Target

Normal arterial structure and function. Throughout the circulatory system, the arterial network combines cushioning (elasticity, mainly mediated by the proximal arteries), and conductance functions, which increase in a stepwise fashion from the aorta to the periphery (166). In large, more elastic arteries, such as aorta and large branches, stiffness is primarily determined by components of the extracellular matrix, which along with the elastin-to-collagen ratio, decrease toward the periphery as arterial stiffness increases. Stiffness of the smaller arteries and arterioles is determined by hypertrophy and smooth muscle tone. Many characteristics can influence arterial stiffness, including endothelial function and NO availability (167). The stiffness of larger arteries also increases in parallel with blood pressure, as a higher distending pressure leads to recruitment of more inelastic collagen fibers (168). Age is an important determinant of elasticity (169), and large artery stiffening is accelerated in black patients (170).

Arterial Stiffness Assessment

Pulse pressure. The pulse pressure (PP) is a crude index of large artery stiffness, but it depends on other factors also (e.g., stroke volume) (171). The pressure wave amplitude, systolic pressure, and PP increase toward the periphery; diastolic and mean pressure do not change significantly (172). Brachial artery pressures only crudely estimate central hemodynamics and tend to be higher than aortic pressures. Central systolic and diastolic pressures are better indices of afterload and coronary perfusion pressure, respectively. Central PP is partially dependent on the elastic properties of the peripheral arteries, as there is a contribution of the reflected wave to this pressure (173). Noninvasive techniques are now available to measure the central PP.

Pulsed wave velocity and augmentation index. During cardiac systole, rhythmic pressure waves are generated, which propagate to the periphery and are reflected backward to the aorta. Accordingly, the pressure waveform arises from the merging of an incident forward traveling wave and a backward one reflected from the periphery (174). Wave reflection occurs at sites of impedance mismatch, often branch points, and is quantified by the augmentation index, which represents the difference between the first and second peaks. Impedance of the elastic arteries is relatively static, but the smaller arteries are more dynamic depending on smooth muscle tone and vessel size. Vasodilation reduces the augmentation index and vasoconstriction increases it (175). Pulsed wave velocity (PWV) is calculated as the distance between 2 sites divided by the travel time of the pulse; a stiff aorta results in higher PWV. Increased PWV produces an earlier wave reflection that arrives in late systole instead of diastole, augmenting the load on the heart. PWV can be assessed by measuring the transit time between the carotid and the femoral artery with mechanotransducers (Complior system, Artech Medical, Pantin, France). Applanation tonometry (SphygmoCor, AtCor Medical, West Ryde, Australia) involves detection and recording of pressure waves from 2 arterial sites using sensitive tonometers. Aortic PWV can be measured with Doppler ultrasonography (176) and magnetic resonance imaging (177,178). Because NO affects the shape and reflection of the arterial wave, endothelial function can be assessed by recording the shape of the arterial waveform after glyceryl trinitrate administration as an endothelium-independent stimulus and salbutamol as an endothelium-dependent agonist (179,180). Glyceryl trinitrate, an NO donor, reduces wave reflection at low doses before any measurable effect on resistance or mean pressure, suggesting that small arteries are more sensitive than resistance vessels (181). Conversely, inhibiting NO production with LG-monomethyl L-arginine increases wave reflection (182).

Prognostic value of arterial stiffness in HF. Arterial stiffness increases with age (169), cardiometabolic abnormalities (183,184), and increased sodium intake (185), all of which are associated with HF. Increased arterial stiffness is associated with LV diastolic dysfunction (186,187) and HF with preserved EF (188,189). Increases in LV end-systolic and arterial elastance occur with aging, particularly in women, and may result in ventricular-vascular stiffening leading to HF with preserved EF (190). Increased PWV and augmentation index are independently associated with systolic and diastolic dysfunction (191–193). Central PP predicts LV hypertrophy and cardiovascular events (194). Increased PP and adverse outcomes have been reported in patients with asymptomatic LV dysfunction as well as overt HF (195,196). Higher PP predicts HF development in elderly patients and predicts mortality and cardiovascular events after myocardial infarction in those with LV dysfunction (197). The relationship between PP and adverse events is independent of mean arterial pressure, suggesting the role of conduit vessel stiffness in HF. As cardiac output falls, neurohumoral activation and vasoconstriction increase resistance vessel tone to maintain mean arterial pressure but also increase vascular smooth muscle mass, tone, and fibrosis, resulting in increased stiffness and PP. A direct relationship between neurohumoral activation and increased carotid stiffness has been seen in HF (198). Although higher PP portends a mortality risk in chronic HF, lower PP seems to predict mortality in acute HF (199).

Strategies to Improve Endothelial Function in HF

Improved endothelium-dependent vasodilation and increased NO bioavailability among HF patients is seen after 4 weeks of an aerobic exercise program (200). Furthermore, improvement in endothelium-dependent vasodilation with exercise training correlates with increased peak oxygen uptake, suggesting that the improved endothelial function contributes to increased exercise capacity after physical training in HF (158). Other therapies that improve HF survival and EF also improve endothelial function (Fig. 4,
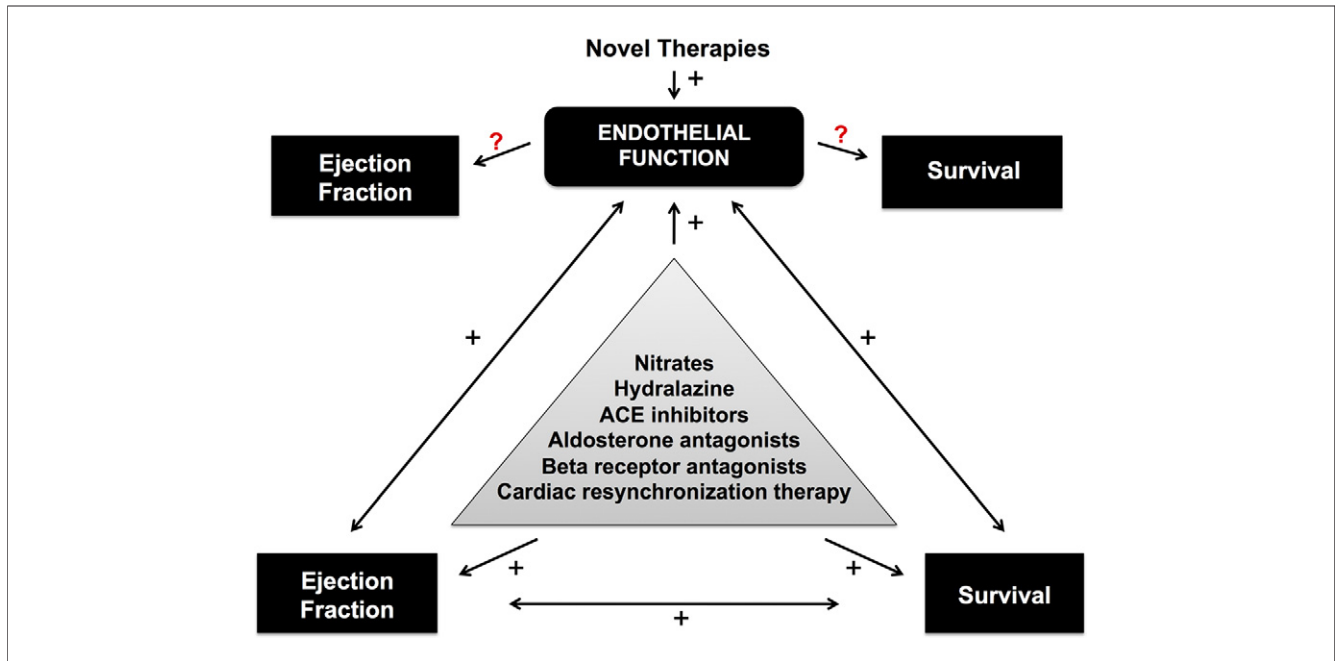
Figure 4 **Effect of Approved Heart Failure Therapies on Ejection Fraction, Endothelial Function, and Survival**

All currently approved therapies for heart failure that have been shown to improve survival and ejection fraction also favorably impact endothelial function. This raises the possibility of assessing endothelial function as a potential early drug development surrogate marker. ACE = angiotensin-converting enzyme.

Table 1 [201–216]). Angiotensin-converting enzyme (ACE) inhibitors improve endothelial function through enhancing bradykinin and reducing oxidative stress (205,217). Addition of spironolactone to an ACE inhibitor exerts additional beneficial effects on endothelium-dependent vasodilation (203,218). Carvedilol, a vasodilating beta-blocker with antioxidant activity, improves oxidative stress (219) and endothelial function (220). Nitrates increase NO bioavailability and affect ventricular remodeling and vascular tone. Hydralazine prevents nitrate tolerance and, through inhibition of reduced nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate oxidase, protects NO from oxidative stress–induced degradation that leads to endothelial dysfunction (221).

Type 5 phosphodiesterase (PDE_5) inhibitors improve NO bioavailability and vasodilation in HF (204). PDE_5 inhibitors increase myocardial contractility (222), blunt adrenergic stimulation (223), reduce LV afterload (222), and improve lung diffusion capacity and pulmonary hemodynamics (224,225). PDE_5 inhibitors have demonstrated improvement in ventilation and aerobic efficiency in HF, which is related to an endothelium-mediated attenuation of exercising muscle oversignaling. The sGC activators and stimulators target the disrupted NO–sGC signaling pathway that affects endothelial function (226). The sGC stimulators sensitize sGC to NO and can stimulate sGC in the absence of endogenous NO, whereas sGC activators activate the NO-unresponsive, heme-free form of the enzyme irrespective of NO bioavailability. Thus, sGC stimulators and activators can treat the 2 forms of sGC insuffi-

ciency (i.e., diminished NO bioavailability and reduction of the catalytic capacity of sGC). Preliminary studies with both PDE_5 inhibitors and sGC-targeted drugs have shown promising results (227–230).

Although most antihypertensive drugs improve arterial stiffness, their beneficial effects on HF may be independent of blood pressure reduction (231). ACE inhibitors favorably affect large- and small-artery elasticity (232) by impeding vascular remodeling and atherosclerosis (231). Some vasodilating beta-blockers also have a favorable effect on the vasculature (233), decreasing stiffness (234). Statin therapy improves arterial elasticity (235) that is related to improved endothelial function and reduced inflammation. Alpha-blockers do not improve arterial stiffness or endothelial dysfunction, even though they lower blood pressure (236).

These associations, although not proven to be causal, nevertheless raise the interesting possibility of targeting endothelial function as a surrogate marker for improved HF outcomes. L-arginine, tetrahydrobiopterin, allopurinol, and progenitor cell therapy are currently under investigation; all favorably influence endothelial function (237,238). Thus, endothelial function is amenable to modulation, providing opportunity for new drug development.

Novel Uses of Endothelial Function Assessment in Phase II HF Trials

Because endothelial function is responsive to both adverse and favorable influences, affects HF, and is measurable, its assessment allows for identification of both positive and

| Table 1 | Effect of Therapeutic Agents on Endothelial Function in HF |

First Author (Ref. #)	Study Population	Therapy (Duration)	Vascular Function	Outcome Measure
Schwarz et al., 1994 (201)	Follow-up study of 18 HF patients and 5 age-matched subjects without HF	Intra-arterial infusion of nitroglycerin (10^{-9} mol/l) (20 min)	Forearm VOP	Forearm blood flow response to acetylcholine increased after administration of nitroglycerin (from baseline reading of 10.6 ± 2.3 to 17.7 ± 3.4 ml/min per 100 ml) in patients with HF but did not appreciably change in normal subjects
Nakamura et al., 1994 (202)	Follow-up study of 30 HF patients	Arterial enalaprilat infusion (0.6 µg/min per 100 ml)	Forearm VOP	Forearm blood flow response to acetylcholine improved after infusion of enalaprilat (2.9 ± 1.1 ml/min per 100 ml)
Farquharson and Struthers, 2000 (203)	Randomized, placebo-controlled, double-blind crossover study of 10 HF patients	Spironolactone 50 mg/day versus placebo (4 weeks)	Forearm VOP	Percentage change in forearm blood flow increased with spironolactone (177 ± 29%) versus placebo (95 ± 20%), with an associated increase in vasoconstriction due to L-NMMA after spironolactone (−35 ± 6%) versus after placebo (−18 ± 4%)
Katz et al., 2000 (204)	Randomized, placebo-controlled, double-blind trial of 48 HF patients	Sildenafil 25 or 50 mg or matching placebo (1 h)	Brachial artery FMD	Percent change in FMD after release of 1, 3, and 5 min of arterial occlusion was greater with sildenafil 25 mg (3.3 ± 1.9%, 3.8 ± 1.8%, and 4.0 ± 1.8%) and 50 mg (3.7 ± 1.3%, 4.1 ± 1.1%, and 3.9 ± 1.3%) than with placebo (0.7 ± 1.1%, 0.2 ± 1.2%, and 0.6 ± 0.8%)
Joannides et al., 2001 (205)	Randomized, placebo-controlled, double-blind trial of 16 HF patients	Perindopril 4 mg/day versus placebo (8 weeks)	Forearm VOP	Flow-dependent dilation and increase in compliance ($3.2 \pm 0.8 \times 10^{-7}$ to $6.8 \pm 2.5 \times 10^{-7}$ m²/kPa) and distensibility ($5.7 \pm 1.4 \times 10^{-3}$ to $8.9 \pm 1.9 \times 10^{-3}$/kPa) of the radial artery was higher with ACE inhibitors
Falskov et al., 2011 (206)	Randomized controlled trial of 27 HF patients	Carvedilol 50 mg/day versus metoprolol tartrate 200 mg/day or metoprolol succinate 200 mg/day (8 weeks)	Forearm VOP	Relative forearm blood flow measured before and after treatment was similar with carvedilol (from 2.4 ± 0.3 to 2.1 ± 0.2 ml/min per 100 ml), metoprolol tartrate (from 2.6 ± 0.3 to 2.4 ± 0.6 ml/min per 100 ml), and metoprolol succinate (from 1.8 ± 0.3 to 2.1 ± 0.4 ml/min per 100 ml)
Doehner et al., 2002 (207)	Randomized, double-blind, crossover study of 19 HF patients	Allopurinol 300 mg/day or placebo (1 week)	Forearm VOP	Percent change in forearm blood flow was higher after allopurinol in arms (25.6 ± 3.5 to 27.8 ± 3.5 ml/min per 100 ml, 24%) and legs (17.4 ± 2.1 to 20.2 ± 2.3 ml/min per 100 ml, 23%) vs. no appreciable change with placebo
Farquharson et al., 2002 (208)	Randomized, placebo-controlled, double-blind crossover study of 11 HF patients	Allopurinol 300 mg/day versus placebo (4 weeks)	Forearm VOP	Percent change in forearm blood flow in response to acetylcholine was higher after allopurinol (181 ± 19%) vs. placebo (120 ± 22%)
Abiose et al., 2004 (209)	Follow-up study of 20 HF patients	Spironolactone (4–8 weeks)	Brachial artery FMD	Percent change in FMD after spironolactone was from 5.5 ± 2.1% to 9.3 ± 4.0% after 4 weeks and 9.0 ± 3.4% after 8 weeks of therapy
Macdonald et al., 2004 (210)	Randomized controlled trial of 43 HF patients	Spironolactone 12.5–50 mg/day versus placebo (12 weeks)	Forearm VOP	Percent change in forearm blood flow response to acetylcholine was significantly improved after treatment with spironolactone vs. placebo (p = 0.045)
Tousoulis et al., 2005 (211)	Randomized follow-up study of 38 male patients with ischemic HF	Atorvastatin 10 mg/day (n = 14), atorvastatin 10 mg/day, and vitamin E 400 IU/day (n = 12) vs. control (n = 12) (4 weeks)	Forearm VOP	Percent change in forearm blood flow in response to reactive hyperemia was higher in the atorvastatin-treated group (from 5.8 ± 2.1 to 6.8 ± 2.4 ml/min per 100 ml) vs. atorvastatin plus vitamin E group (from 5.6 ± 1.6 to 6.0 ± 2.1 ml/min per 100 ml) and control group (from 5.5 ± 2.0 to 5.7 ± 2.2 ml/min per 100 ml)
George et al., 2006 (212)	Randomized, placebo-controlled, double-blind, crossover study of 30 patients with HF	Allopurinol 300 mg/day or 600 mg/day versus placebo (4 weeks)	Forearm VOP	Percent change in forearm blood flow in response to acetylcholine was higher after allopurinol 600 mg/day (240.3 ± 38.2%) compared with both allopurinol 300 mg/day (152.1 ± 18.2%) and placebo (74.0 ± 10.3%)
Guazzi et al., 2007 (213)	Randomized controlled trial of 46 patients with HF	Sildenafil 50 mg twice per day (6 months)	Brachial artery FMD	Percent change in FMD with sildenafil was higher (8.5% to 13.4% and 14.2% at 3 and 6 months, respectively) vs. placebo (from 7.8% to 7.6% and 8.1% at 3 and 6 months)
Castro et al., 2008 (214)	Prospective study of 38 patients with HF	Atorvastatin 20 mg (8 weeks)	Brachial artery FMD	Percent change in FMD was higher after therapy with atorvastatin (from 4.5 ± 1.9% to 6.7 ± 2.8%) vs. placebo (from 4.5 ± 1.9% to 5.0 ± 2.0%)
Gounari et al., 2010 (215)	Double-blind, placebo controlled, crossover trial of 22 patients with HF	Ezetimibe 20 mg or rosuvastatin 10 mg (4 weeks, with a 4-week washout period)	Brachial artery FMD	Percent change in FMD after therapy with rosuvastatin was significantly higher (p < 0.05 versus baseline), whereas there was no change after ezetimibe treatment (p = NS vs. baseline)
Erbs et al., 2011 (216)	Randomized, double-blind, placebo-controlled study of 42 HF patients	Rosuvastatin (40 mg/day) or placebo (12 weeks)	Brachial artery FMD	Percent change in FMD after therapy with rosuvastatin (163%, p < 0.001 vs. placebo)

ACE = angiotensin-converting enzyme; FMD = flow-mediated dilation; HF = heart failure; L-NMMA = N-monomethyl-L-arginine; VOP = venous occlusion plethysmography.

negative drug effects. Endothelial function assessment may offer advantages over other Phase II trial surrogate end points (hemodynamic or symptom based) by providing mechanistic insights into investigational therapies. The additional endothelial function assessment will be complementary to hemodynamic, imaging, and symptom-based endpoints, and positive findings may provide a firm rationale for prioritization of drugs for testing in large-scale outcome studies. Efforts to standardize endothelial function assessment, (e.g., FMD) have improved reproducibility, and both crossover and parallel design clinical trials have become feasible and published power curves facilitate protocol design (239). Endothelial function assessment may improve classification of HF pathophysiology as well. This is important given the critical need for improved categorization of the HF syndromes (240). This may also reduce patient heterogeneity in clinical trials. By providing mechanistic insights in Phase II studies, endothelial function and arterial stiffness assessments may further inform subsequent phases of drug development, provide the rationale to re-examine preclinical models, develop new uses for investigational agents, and better determine which patients may benefit most in Phase III trials.

Conclusions

Endothelial dysfunction is implicated in HF development, is prevalent in those with HF, is associated with HF progression, and is a predictor of adverse events in these patients. Specific techniques can be used to evaluate coronary and peripheral conductance and resistance vessel endothelial function. Similarly, arterial stiffness may be related to and exacerbate HF, especially with preserved EF. These techniques have a firm theoretical basis and address different facets of endothelial and vascular physiology. Evaluation of endothelial function and vascular status may be a valuable mechanistic surrogate that could aid novel therapeutic drug development. It is important, however, to perform studies that address relevant questions, including which techniques are most informative in HF and whether the clinical benefit from a specific therapeutic strategy is mediated through an improvement in endothelial function.

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