Mechanisms of Diastolic Dysfunction in Heart Failure With a Preserved Ejection Fraction
If It’s Not One Thing It’s Another

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It has been nearly 30 years since the first series of patients with the syndrome of heart failure with a preserved ejection fraction (HFpEF) was reported. It has proven to be a controversial topic. Because left ventricular (LV) EF is preserved, it was assumed that HFpEF results from altered diastolic properties. However, some argued that these patients did not truly have HF or had subtle forms of dilated HF. Symptomatic of this debate is reluctance to use the term diastolic HF (we prefer HFpEF because diastolic dysfunction is also present in HF with a reduced EF) as well as disagreement over the exact EF cutoff, that is, should a perfectly normal EF be required to diagnose HFpEF or does a modest reduction qualify?

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Although many questions remain, in the intervening years several features have emerged. HFpEF is a complex and extremely common syndrome, accounting for >50% of patients with HF. It is more prevalent in women, and its prognosis is similar to HF with a reduced EF. The clinical presentation ranges from dyspnea with physical activity to a pattern of restrictive cardiomyopathy, with marked elevations of right and left filling pressure at rest, often with considerable pulmonary hypertension (HTN). Essentially all patients with HFpEF have diastolic dysfunction, specifically, reduced LV passive compliance and/or slowed or incomplete relaxation. Various other cardiovascular abnormalities are common, including subtle abnormalities of systolic function.

HFpEF Substrates

Although a small number of patients have HFpEF in association with specific cardiac diagnoses, for example, hypertrophic and infiltrative cardiomyopathy, constrictive pericarditis, all of which have profound effects on diastolic compliance, the vast majority have a history of HTN. In many patients, especially elderly women, HTN is exclusively systolic, resulting from reduced arterial compliance rather than changes in resistance vessels. Moreover, although there is considerable variation between patient cohorts, the great majority of patients with HTN-associated HFpEF have concentric LV remodeling, defined as either concentric hypertrophy (increased LV mass with normal or reduced chamber volume) or, in the absence of increased mass, increased mass:volume ratio or relative wall thickness. In population studies, the progression from HTN to HFpEF is paralleled by declines in diastolic function. These observations strongly support the concept that diastolic dysfunction is in fact a major underlying mechanism of this progression, resulting in the hemodynamic hallmark of HF, a depressed Frank–Starling relation.

HTN is not the only substrate in many if not most patients with HFpEF. Approximately one third have type 2 diabetes mellitus (DM2). It is likely that a substantial additional number have insulin resistance in the absence of overt DM2. Insulin resistance/DM2 and associated hyperinsulinemia have pleiotropic effects on the myocardium, including stimulation of hypertrophy, increased oxidative stress, and a proinflammatory/profibrotic state, which can modify cardiomyocyte function in multiple ways as well as extracellular matrix collagen, all of which can affect diastolic function. Obstructive sleep apnea and obesity are common in HFpEF and also associated with a proinflammatory state and cardiac hypertrophy. HTN, DM2/insulin resistance, and obesity are components of the metabolic syndrome (MS). Recognition of the association between MS and HFpEF has led to the concept that in many patients HFpEF can be considered metabolic heart disease, although the detailed mechanisms whereby metabolic derangements and associated oxidative stress and proinflammatory/profibrotic states cause diastolic dysfunction remain to be elucidated. Abnormal myocardial triglyceride accumulation associated with echocardiographic evidence of diastolic dysfunction in patient with elements of the MS provides direct evidence of this link.

Mechanisms of Diastolic Dysfunction in HFpEF

The exact mechanisms leading to diastolic dysfunction in concentric remodeling and HFpEF have begun to be elucidated during the past 5 to 10 years. In discussing the article by Hamdani et al in this issue of Circulation: Heart Failure, we will focus on its relationship to what is known about these mechanisms from studies on myocardial tissue from patients.

One well-documented mechanism studied in biopsy tissue from patients with HFpEF is hypophosphorylation of protein kinase (PK) A and PKG sites on cardiac titin, the giant myofilament protein responsible for cardiomyocyte passive tension. Titin’s N terminus is anchored in the z-disc of the sarcomere, and its C terminus is anchored in the M-band. When the cardiomyocyte is stretched, titin lengthens and...
functions as a complex molecular spring, developing passive tension with a curvilinear length–tension relationship. Chemical disruption of titin’s anchors in the M-band eliminates virtually all cardiomyocyte passive stiffness over the physiological sarcomere length range. Using these chemical methods, the proportion of myocardial passive tension ascribable to titin versus extracellular matrix collagen has been dissected. Although there are differences in absolute levels of passive tension, in all species studied, including humans, titin accounts for the majority of myocardial passive tension at short sarcomere lengths. With further lengthening, the relative contribution of collagen increases such that it accounts for ≈50% or more of passive tension at sarcomere lengths at the upper end of the physiological range. Titin is also a key biomechanical sensing and signaling molecule and the most commonly mutated gene in human dilated cardiomyopathy. These and other functional and disease-specific aspects of titin have been discussed in recent reviews.

Titin stiffness is modulated by isoform variation accomplished by alternative splicing and changes in phosphorylation state. Two isoforms (N2B and N2BA) are present in the postnatal heart; N2B is smaller and markedly stiffer than N2BA. The N2BA:N2B ratio is ≈40:60 in normal adult human LV myocardium. In both ischemic and nonischemic dilated cardiomyopathy as well as HfPEF, a shift toward the more compliant N2BA isoform occurs, which reduces cardiomyocyte resting tension. Changes in phosphorylation can rapidly alter myocardial passive stiffness, for example, during exercise. In HfPEF, the net effect of increased N2BA titin and hypophosphorylation of PKA/PKG sites is increased cardiomyocyte resting tension. In addition to PKA/PKG, PKC-α phosphorylate multiple, identical sites on titin, which reduces cardiomyocyte resting tension. Changes in phosphorylation can rapidly alter myocardial passive stiffness, for example, during exercise. In HfPEF, the net effect of increased N2BA titin and hypophosphorylation of PKA/PKG sites is increased cardiomyocyte resting tension. In addition to PKA/PKG, PKC-α has been shown to phosphorylate other titin sites. CaM kinase targets these same sites. In contrast to PKA/PKG sites, PKC-α phosphorylation increases resting tension.

In their elegant study, Hamdani et al used Zucker rats to demonstrate that the combination of obesity, DM, and HTN (with or without a high-fat diet) leads to HfPEF in association with increased passive myocardial stiffness and markedly reduced phosphorylation of titin’s PKA/PKG sites compared with controls. There were no changes in isoforms or phosphorylation of one of the PKC-α sites. Importantly, phosphorylation of PKA/PKG sites was unchanged in lean, nondiabetic, but hypertensive Zucker rats. Thus, components of the MS besides HTN seem sufficient to cause changes in passive stiffness attributable to reduced titin phosphorylation in this experimental model. Although the underlying mechanism(s) of reduced phosphorylation was not elucidated, this article provides important insights into the pathophysiology of HfPEF that could play a role in patients.

Hamdani et al have not shown that reduced titin phosphorylation is sufficient in and of itself to cause HfPEF in obese–diabetic–hypertensive rats. Lean hypertensive rats developed significant but modest increases in LV mass at the last, 18th week, measuring point, which were not associated with changes in diastolic function indexes. In contrast, increases in mass were much larger and occurred much earlier in obese–diabetic–hypertensive rats and were associated with abnormal diastolic function. Thus, it is important to consider determinants of diastolic function other than titin that could contribute to the development of diastolic dysfunction and HfPEF.

A modest amount of such information obtained in human tissue is now available, although it has not been specifically focused on metabolic heart disease. One determinant is changes in extracellular matrix collagen, but Hamdani et al report that collagen volume fraction and cross-linking were unchanged. However, this differs from HfPEF in patients, in whom collagen volume fraction and cross-linking are increased and underscores the potential for animal models to provide information that does not apply to patients. Using the chemical methods noted above, Hamdani et al also report that collagen-dependent passive tension was unchanged. However, an unexplained finding is that collagen-dependent tension accounted for only ≈10% to 20% of total passive tension in all groups. As noted above, this is much smaller than what has been reported previously in several species.

In addition to passive diastolic properties, LV relaxation is abnormal in patients with LV hypertrophy and HfPEF, but the mechanisms have received less attention. At the level of the LV, increased arterial load, when present, slows relaxation rate. At the myocardial level, the speed and completeness of relaxation are dependent on deactivation of cross-bridges formed during contraction, which in turn depends on both the mechanisms that restore systolic [Ca2+]i to diastolic levels and the kinetics of cross-bridge dissociation.

We recently reported the first evidence of abnormal calcium handling in patients with pressure overload–induced concentric remodeling. In excitable tissue from LV epicardial biopsies obtained from patients with normal EF undergoing coronary bypass grafting, we found that isometrically contracting strips from patients with concentric remodeling (some of whom had HfPEF) displayed a progressive increase in diastolic tension beginning at stimulation frequencies in the 100 to 110 per minute range, that is, incomplete relaxation occurred at rates present during low-level physical activity. Additional experiments revealed a defect in sarcolemmal calcium extrusion. In more recent, unpublished work, we found that cytoplasmic [Ca2+]i is indeed increased at these same rates. These results may provide a mechanism whereby patients with HfPEF increase filling pressures and become dyspneic with physical activity. Correspondingly, patients with HfPEF display a reduced ability to maintain end-diastolic volume and cardiac output during increases in heart rate, which could reflect the same mechanism.

In another recent report using demembranated (skinned) myocardial strips, we showed that the kinetics of cross-bridge dissociation are slowed in patients with concentric remodeling compared with controls. Using the method of sinusoidal length perturbation, the apparent rate constant of cross-bridge dissociation was reduced at submaximal [Ca2+]i and its mathematical inverse, cross-bridge on-time (the time the cross-bridge is attached and generating force) was prolonged. These changes in dissociation kinetics serve to slow relaxation. We also found that total phosphorylation of both cardiac troponin I and myosin binding protein C is reduced in concentrically remodeled LV myocardium. Recent, unpublished studies using site-specific phosphoantibodies reveal...
that PKA/PKG sites on both proteins are hypophosphorylated. Because phosphorylation of these sites speeds actomyosin kinetics, hypophosphorylation may contribute to slowed relaxation.

In summary, although studies are limited, in patients with HFpEF or pressure overload–induced concentric remodeling abnormalities of every component of LV diastolic function, arterial load, mass:volume ratio, passive stiffness (titin and collagen), and cross-bridge deactivation (calcium handling and actomyosin kinetics) have been demonstrated or implicated. In future, it will be important to understand the relative importance and time course of these abnormalities in relation to the progression to HFpEF as well as the influence of substrates other than HTN.

Therapeutic Considerations
There are currently no therapies for HFpEF that have been shown to improve long-term outcomes. Perhaps the diverse abnormalities of diastolic function identified, which could have great interpatient variability, make it difficult for treatments to yield significant effects in clinical trials. Guidelines for treatment are, therefore, largely empirical, emphasizing Na restriction, diuretics as needed, and blood pressure control. In patients with HFpEF with MS, common sense suggests that weight loss and perhaps exercise should be therapeutic goals. Small trials show that weight loss can improve diastolic function, but the effects of exercise have been variable. The study by Hamdani et al17 links a specific component of diastolic dysfunction in HFpEF, that is, titin hypophosphorylation, to DM2 and obesity and suggests 1 potential mechanism whereby lifestyle changes can improve diastolic function.

Our knowledge of the mechanisms of diastolic dysfunction in concentric remodeling and HFpEF, although admittedly rudimentary, has other therapeutic implications. It is intriguing that a specific alteration at the level of the myofilaments, hypophosphorylation of PKA/PKG sites, may contribute to increased passive stiffness (titin) and slowed relaxation (cardiac troponin I/myosin binding protein C). Accordingly, pharmacological approaches that target this molecular abnormality offer promise. Unfortunately, the RELAX (Phosphodiesterase-5 Inhibition to Improve Clinical Status and Exercise Capacity in Heart Failure with Preserved Ejection Fraction) trial of sildenafil in HFpEF31 did not demonstrate efficacy despite the fact that phosphodiesterase-5 inhibition has several effects that, in addition to potential normalization of titin and cardiac troponin I/myosin binding protein C phosphorylation, should be beneficial.32 In the RELAX trial, sildenafil did not significantly increase plasma cGMP activity, suggesting that PKG activity may not have been effectively augmented. This in turn suggests that other approaches to increasing NO availability and PKG activity should be considered. Nitrates are an obvious choice. Other considerations arise concerning exercise and the common use of β-blockers in HFpEF. Hypophosphorylation of PKA/PKG sites should be ameliorated during exercise in conjunction with increased adrenergic stimulation, that is, their importance may decrease with physical activity. β-Blockers could potentiate these same abnormalities at rest and during exercise. In contrast, rate-dependent incomplete relaxation and inadequate maintenance of end-diastolic volume are obviously more pronounced during exercise and could therefore be more important as a mechanism of exercise limitation. In that case, β-blockers may help by blunting increases in heart rate during exercise. These divergent heart rate effects might make it difficult to detect beneficial effects of β-blockers.

Targeting the extracellular matrix is obviously also a promising therapeutic approach. Aldosterone inhibition is potently antifibrotic and has other potentially beneficial effects.33 The recent Aldo-DHF (Aldosterone Receptor Blockade in Diastolic Heart Failure) phase 2 trial of spironolactone33 revealed improvements in resting diastolic function in HFpEF. The results of the larger, ongoing TOPCAT (Treatment of Preserved Cardiac Function with an Aldosterone Antagonist) trial of spironolactone in HFpEF34 are therefore eagerly awaited.

Summary
We are beginning to gain a better understanding of the mechanisms of diastolic dysfunction in HFpEF. By demonstrating reduced titin phosphorylation in obese–diabetic–hypertensive rats, Hamdani et al17 have provided important insights into the substantial number of patients with HFpEF with components of the MS, that is, metabolic heart disease. As progress is made in other mechanistic aspects of HFpEF, we will hopefully gain a more integrated understanding and a more rational basis for developing new treatments. In view of the multiple abnormalities of diastolic function identified, it may be particularly important to individualize and target treatment.

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References


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