Mechanisms of Diastolic Dysfunction in Cardiovascular Disease

Myocardial reverse remodeling: how far can we rewind?

Patrícia G. Rodrigues, Adelino F. Leite-Moreira, and Inês Falcão-Pires

Department of Physiology and Cardiothoracic Surgery, Faculty of Medicine, Universidade do Porto, Porto, Portugal

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Heart failure (HF) is a systemic disease that can be divided into HF with reduced ejection fraction (HFrEF) and with preserved ejection fraction (HFpEF). HFpEF accounts for over 50% of all HF patients and is typically associated with high prevalence of several comorbidities, including hypertension, diabetes mellitus, pulmonary hypertension, obesity, and atrial fibrillation. Myocardial remodeling occurs both in HFrEF and HFpEF and it involves changes in cardiac structure, myocardial composition, and myocyte deformation and multiple biochemical and molecular alterations that impact heart function and its reserve capacity. Understanding the features of myocardial remodeling has become a major objective for limiting or reversing its progression, the latter known as reverse remodeling (RR). Research on HFrEF RR process is broader and has delivered effective therapeutic strategies, which have been employed for some decades. However, the RR process in HFpEF is less clear partly due to the lack of information on HFpEF pathophysiology and to the long list of failed standard HF therapeutics strategies in these patient’s outcomes. Nevertheless, new proteins, protein-protein interactions, and signaling pathways are being explored as potential new targets for HFpEF remodeling and RR. Here, we review recent translational and clinical research in HFpEF myocardial remodeling to provide an overview on the most important features of RR, comparing HFpEF with HFrEF conditions.
geting comorbidities) and early diagnosis of HFpEF; however, the lack of sufficient understanding on the mechanisms driving LV remodeling in HFpEF represents a major limitation. Nevertheless, considering that myocardial remodeling is clearly dissimilar between HFpEF and HFrEF, it is thus conceivable that these two entities also present distinct reverse remodeling (RR) processes.

From an historical perspective, the term “ventricular remodeling” became popular in the mid-1980s, with the study of Pfeffer (189), who described it as a progressive LV dilatation and diminished function, after coronary occlusion in rats (166, 188). Over the years the term was used for virtually anything that was altered as a result of HF or a result of any other cardiac disease that spans maladaptation at molecular, cellular, tissue, and whole organ levels (38, 184). Nowadays, it is recognizable that these studies were only focusing on HFrEF.

In turn, definition of “reverse remodeling” dates back to a study of cardiac myoplasty (115) in which the latissimus dorsi muscle was wrapped around a heart and paced synchronously with ventricular systole in an effort to enhance systolic function. One year after cardiac myoplasty, all patients showed a reduction in chamber volume and steeper end-systolic pressure-volume relation, allowing the ventricle to generate higher systolic pressure at a given LV volume and indicating that the heart had shrunk towards normal size. A key feature of this adaptation was that the geometric change persisted, even if therapy was turned off abruptly, and was thus intrinsic to the chamber. As with the term “remodeling,” “reverse remodeling” also became associated with any alteration in HF that could be chronically reversed by a given therapy, such pharmacological therapy, valve surgery (e.g., aortic stenosis) or assist devices (e.g., chronic HF). “Myocardial recovery” is another term widely used to describe the changes in HF after medical treatment (261). However, Mann and et al. (160) suggested that myocardial recovery is a different phenomenon, which is normally associated with no future failure events after HF treatment. Despite all efforts to understand RR in HF, it remains to clarified which of its components are necessary to accomplish myocardial recovery.

Regarding HFrEF, research on its RR is broader with effective therapeutic strategies being employed for several decades based on a deeper knowledge of its pathophysiologic mechanisms (Fig. 1). These include inhibitors of the renin-angiotensin-aldosterone system (RAAS), β-blockers, and device therapies such as cardiac resynchronization therapy or left ventricular assist devices (LVAD), although not every method aiming for RR has shown long-lasting clinical efficacy (129). In general, cardiac RR in HFrEF, at the cellular level, is charac-
terized by changes in cardiomyocyte size, function, excitation-contraction coupling, and bioenergetics and by “normalization” of molecular pathways that regulate contraction, cell survival, mitochondrial function, oxidative stress, and other features (129). Studies on LVAD patients have contributed significantly to the current knowledge of RR in HFrEF, since it allows the collection of myocardial tissue in the same patient before and after RR, during implantation and removal of the LVAD, respectively.

In this review, we will provide an integrated view of the continuum of changes taking place in myocardial RR, given focus to left ventricular hypertrophy (LVH) and fibrosis and diastolic dysfunction regression, important features of HFrEF pathophysiology. This review will briefly describe myocardial remodeling as the basis to provide an overview on the most important features of RR, comparing HFrEF with HFrEF conditions. Moreover, there is currently a scarceness of studies focusing on HFrEF RR. Thus we will emphasize RR of human and experimental pressure overload studies as these conditions usually progresses to concentric LVH and diastolic dysfunction. Despite the reduced amount of information, whenever possible, we will emphasize RR in HFrEF patients whose pathophysiology has remained obscure due to the absence of a proper animal model and to the confounding effects of other prevalent comorbidities.

Myocardial Remodeling

The process of myocardial remodeling is influenced by hemodynamic load, neurohumoral activation, and other factors still under investigation (38). As the heart remodels, its geometry and ventricular mass changes in parallel with important cellular and molecular modifications, including cardiomyocyte size and shape changes, excitation-contraction coupling, cell-survival signaling and metabolic disturbances, and, in some animal models, reexpression of fetal gene program. In parallel, extracellular matrix (ECM) remodeling also occurs, triggering cardiac chamber deformation and alterations in the composition of fibrous and vascular elements in the myocardium (129). Despite that the first studies on remodeling were carried out in models of systolic dysfunction or HFrEF, currently a lot of effort has been gathered to understand the pathophysiology driving myocardial remodeling in HFrEF. Indeed, it is currently accepted that HFrEF and HFrEF remodeling processes are distinct (Fig. 1) (97). In this section we will briefly described some of the principal contributors for myocardial remodeling, focusing on LVH, ECM abnormalities, myocardial cell death, and metabolic disturbances and emphasizing the major differences between HFrEF and HFrEF. Detailed information on HFrEF and HFrEF myocardial remodeling is described in Refs 1, 31, and 27, respectively.

LVH, as assessed by increased LV mass, has long been regarded as a compensatory response to preserve LV function under overload conditions. Contrary to the reversible physiological hypertrophy, induced by exercise training or pregnancy, chronic overload induced by conditions such as hypertension, coronary artery disease, or valvular heart disease leads to pathological hypertrophy. Reversal of myocardial hypertrophy in the pathologic condition is still a matter of debate, and the differences with the pathologic scenario remain controversial (184, 260). LVH can be classified into three groups: concentric hypertrophy (increased relative wall thickness and normal internal diameter), eccentric hypertrophy (increased relative wall thickness and increased internal diameter), and concentric remodeling (enlarged heart with normal relative wall thickness). While the first frequently develops in HFrEF patients, the second is more associated with an HFrEF phenotype and the latter with the RR process (75, 97). At the cellular level, the major differences between eccentric and concentric hypertrophy rely on the 1) shape of cardiomyocytes (long and thin vs. short and enlarged); 2) organization of sarcomeres (“in series” vs. “in parallel”) (128); and 3) mechanisms controlled by different proliferative signaling pathways (129). LVH itself is a major independent risk factor for cardiovascular morbidity and mortality (143). Specifically, in HFrEF, loss of cardiomyocytes (e.g., acutely with myocardial infarction or chronically with idiopathic cardiomyopathy) often results in left ventricular eccentric hypertrophy and cardiac failure. Contrarily, in HFrEF, concentric myocardial hypertrophy, as a result of multiple cardiovascular risk factors, imposes a bad prognosis since it is predictive of higher HF hospitalizations, cardiovascular death or sudden cardiac arrest (210), and reduced exercise capacity (168). In fact, an echocardiographic substudy of the Irbesartan in Heart Failure with Preserved Systolic Function (I-PRESERVE) trial including patients with HFrEF demonstrated a high prevalence of structural remodeling, with 59% having LVH in a pattern of concentric remodeling (281). However, eccentric hypertrophy can also occur in HFrEF patients, indicating a distinct subgroup of patients that may progress to HFrEF (117). In HFrEF clinical settings, as opposed to many experimental models, the transition to HFrEF is rarely observed (97) but, when it takes place, this transition is due to loss of contractile function within the remaining cardiomyocytes during LV remodeling, which is in line with distinct signaling pathways between HFrEF and HFrEF (97, 171).

Although adaptive in the early stages, LVH eventually becomes maladaptive and contributes to the development of diastolic dysfunction (97). For instance, in chronic pressure overload associated with HFrEF, the development of LVH is simultaneous with remodeling of the ECM with progressive interstitial fibrosis and reduced ventricular compliance and diastolic dysfunction (80). Diastolic abnormalities, such as slow LV relaxation and elevated diastolic LV stiffness, are the most common finding in HFrEF patients at rest (1). The increased diastolic wall stiffness impairs cardiac relaxation and filling, decreases end-diastolic volume, and increases end-systolic pressure, resulting in a left and upward shift of the LV end-diastolic pressure-volume relation. Consequently to long-standing increased ventricular filling pressures, atrial enlargement, reflecting the degree of structural remodeling (113), occurs in 85% of HFrEF patients as demonstrated in I-PRESERVE trial (281). Indeed, moderate or severe diastolic dysfunction is a predictor of cardiovascular death or HF hospitalization in the HFrEF population (187). Nevertheless, the underlying pathomechanisms that may link LVH to diastolic dysfunction and HFrEF are not yet completely understood. In this regards, it was proposed that higher levels of cytosolic Ca2+ may contribute to slowed myofilament relaxation during diastole (153). In addition, hypophosphorylation of myofilaments leading to increased Ca2+ sensitivity may also contribute to impaired cardiomyocyte relaxation in HFrEF (91).
In a mouse model, microvascular rarefaction preceding LVH suggested that microvascular dysfunction may also be a cause of diastolic dysfunction independently of LVH (186). LVH, as well as other risk factors associated with HFpEF such as age, diabetes, obesity, and hypertension, have been linked to coronary microvascular rarefaction in animal models and patients. Recently, Lee et al. (142) investigated vascular function in patients with HFpEF at the conduit and microvascular levels and identified a distinct pattern of vascular dysfunction that is specific to the microvasculature. Microvasculature impairment may be of particular importance in the context of coronary circulation (142). This was recently reinforced by Paulus and Tschope (185). These authors proposed that coronary microvascular dysfunction in HFpEF results in changes in ECM composition and cardiomyocytes function (185). This topic will be emphasized in Altered Signaling Pathways in HFpEF as Potential Targets for Reverse Remodeling.

Modifications in ECM constitute the second most important myocardial adaptation that occurs during HFpEF and HFrEF remodeling. These include changes in overall and relative subtypes of collagen content, collagen cross linking, and connections between cells and the ECM via integrins (160). The presence of stress, leads to activation of fibroblasts triggering the disproportionate synthesis of collagen, fibronectin, and laminin. Specifically in HFpEF, several stimuli contribute to myocardial fibrosis (79) including cytokines [transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), and interleukin family], angiotensin II, aldosterone, endothelin-1 (ET-1), and cathecolamines (30, 121, 205, 227). Collagen turnover is mainly regulated by matrix metalloproteinases (MMPs), as their expression and activation are significantly upregulated in pathological LVH (154). Nonetheless, the net deposition of fibrillar collagens prevails due to the production of tissue inhibitor of MMPs (TIMPs) associated with the increase in MMPs. The lower MMPs/TIMPs ratio favors excessive fibrosis and consequently compromises ventricular filling (36). Contrarily, LV biopsies from HFrEF patients displayed a decrease in fibrosis due to increased ECM degradation, which was related to upregulation of MMPs (MMP-9, -2, and -3) (221), providing evidence of the distinct pattern of ECM changes underlying HFpEF and HFrEF.

Intrinsic cardiomyocyte changes also contribute to myocardial remodeling. For instance, the cytoskeletal protein titin is the main determinant of LV stiffness within physiological sarcomere lengths (SL: 1.8–2.2 μm), accounting for ~80%, while the contribution of the ECM only becomes crucial at a SL higher than 2.2 μm (148). Alterations in cardiac titin isoform expression have been found in patients with HFpEF, wherein the N2BA:N2B expression ratio was decreased compared with HFrEF (243). Contrarily, in dilated explanted hearts or ischemic cardiomyopathy patients, the N2BA:N2B expression ratio is increased compared with normal hearts, resulting in reduced titin-based myocardial stiffness (177). Besides the long-term changes in the isoform ratio that accompany chronic cardiovascular disease, titin stiffness is also amenable to modulation by phosphorylation (24, 92, 99, 133, 134, 244) or by oxidative stress-induced formation of disulphide bridges within the titin molecule (87). We have gathered a substantial amount of data showing titin hypophosphorylation, both in failing human myocardium (22) and in an animal model of HFpEF (92). Studies carried out in an animal model of HFpEF described titin hypophosphorylation, which importantly contributed to the higher stiffness of cardiomyocytes and consequently to the diastolic dysfunction observed in these animals (92). Also, an animal model of HFrEF induced by volume overload presented decrease levels of titin phosphorylation. In this case, the hypophosphorylation of titin represented a compensatory mechanism to the lack of cardiac interstitial fibrosis and cardiomyocytes loss in disease animals (171).

Cardiac cell loss, also observed in myocardial remodeling, typically results from exaggerated autophagy, apoptosis, or necrosis under conditions of injury-induced ventricular remodeling, such as in severe pressure overload-induced hypertrophy (85, 175), ischemic injury, or postmyocardial infarction (157) and in HFrEF patients (182). This feature is more closely associated with HFrEF rather than HFpEF remodeling (Fig. 1). In HFrEF, the excessive wall stress, triggered by cardiomyocyte loss shifts the balance in the ECM between collagen deposition and degradation, leading to the appearance of patchy areas of fibrosis. This phenomenon importantly contributes to HFrEF typical LV dilation and eccentric remodeling (185). In fact, a study using endomyocardial biopsy samples from HFrEF showed higher levels of patchy interstitial fibrosis compared with HFpEF patients biopsies (243, 245). Apoptotic cardiomyocyte death, oxidative stress (48), and metabolism abnormalities (110) were also related to the appearance of eccentric hypertrophy in an animal model of transverse aortic constriction (TAC), which initially triggers concentric hypertrophy. In humans, this progression is observed mostly in myocardial infarction patients (185).

Abnormalities in cardiac energy metabolism occur in parallel with the development of LVH and myocardial remodeling (110). Under normal conditions, the oxidation of fatty acids is the major pathway, providing about 70% of the total energy demand, while glucose oxidation provides the rest (264). It is well known that depletion of myocardial energy reserve and mitochondrial dysfunction represents a major cause of dysfunction of the failing human heart (179). In fact, decreased fatty-acid oxidation and increased glucose utilization are observed in pathological hypertrophic and HFrEF (242). This shift enhances the glycolytic pathway, thus increasing anaerobic metabolism (242) and oxidative stress by increased reactive oxygen species (ROS) production. The latter mechanism is known to damage mitochondria inducing, for instance, mitochondrial permeability transition pore opening (60) and consequently cell death (132). Also, mitochondrial DNA damage leads to deficient functioning components of the cellular energetics machinery, thus directly contributing to increased oxidative stress underlying cardiac hypertrophy and HFrEF (44). In the healthy heart, buffering systems, such as the creatine kinase reaction, maintain adenosine triphosphate (ATP) levels (179). However, in pathological conditions, namely in HFpEF, a reduced energy reserve, as assessed by the phosphocreatine/adenosine triphosphate (PCr/ATP) ratio, is observed mostly due to PCr drop (>50%) (4). The principal consequence of low PCr and/or a reduced creatine kinase activity is the rise of cytosolic adenosine diphosphate (ADP) that was linked to severe LVH in a canine pressure overload model (110) and to increased LV end-diastolic pressure (234). Moreover, changes in relaxation were associated with mitochondrial functional disability in ATP production (66). This supports the idea that limited myocardial energy reserve via elevations of ADP is
likely a cause of myocardial diastolic dysfunction. Indeed, Sequeira et al. (209) recently provided evidence that conditions that elevate myocardial ADP in the presence of diastolic Ca\textsuperscript{2+} contribute to diastolic dysfunction by increasing residual actomyosin interactions. The authors showed in rats that physiological ADP levels found in diseased hearts (100 \textmu M) increase Ca\textsuperscript{2+} sensitivity and stiffness in membrane-permeabilized cardiomyocytes, limit diastolic sarcomere relengthening associated with high Ca\textsuperscript{2+}-buffering of intact cardiomyocytes, and reduce ventricular compliance in isolated Langendorff-perfused hearts (209). The changes in myocardium metabolism and the structural and functional mitochondrial abnormalities reflects on energy production and oxygen consumption (224), which will eventually culminate in lower capacity to perform work. The clinical evidence of these disturbances is confirmed by the exercise intolerance observed in HfPEF patients, measured by a decrease in peak oxygen consumed during maximal effort exercise (VO\textsubscript{2 peak}) (241). Impaired VO\textsubscript{2} is associated with decreased cardiac output, which was, in part, related to chronotropic incompetence (25), impaired systolic reserve (26), and abnormal ventricular-vascular coupling (118) in HfPEF patients. However, the role of these alterations in myocardial metabolism remains controversial, specifically, because it has not been possible to determine whether the metabolic alterations have been a primary or an epiphenomenon (50).

**Myocardial “Reverse Remodeling”**

A variety of cardiac pathologies, including ischemic disease, hypertension, valvular diseases, and genetic forms of cardiomyopathies can lead to extensive myocardial remodeling and eventually to HF. Myocardial maladaptive remodeling is an important aspect of disease progression and its prevention or reversal is a desired strategy. Myocardial remodeling can be reversed completely upon treatment, while in other cases RR is incomplete and the underlying reasons remain to be clarified. In clinical practice, changes in ejection fraction, LV end-diastolic and end-systolic volumes, mass, and sphericity index are used as surrogate parameters for remodeling or RR. In some circumstances (if myocardium is available at different time points), remodeling may also be assessed on the cellular levels. In fact, the majority of the knowledge about cellular and molecular alterations during the RR process comes from studies with LVAD in which myocardial tissue is available before and after unloading of the heart. Besides LVAD, different drugs [e.g., \beta-blockers and angiotensin II-converting enzyme inhibitors (ACEI)] and interventions (ventriculoplasty and resynchronization therapy) have been demonstrated to induce myocardial RR (on the basis of organ-level geometry) or improve clinical outcome, or both, primarily in HFrEF (129). However, in HfPEF less is known about myocardial RR, since its pathophysiology remains obscure, due to the absence of a proper animal model and to the confounding effects of coexisting comorbidities.

In HFrEF, the severity of LV remodeling predicts the response to treatment and patient outcomes. Data from the Framingham study demonstrated that patients, without myocardial infarction but with cardiac dilation, had a 1.47-fold risk of developing HF compared with those without dilation (249). In myocardial infarction, patients with highest ventricular volumes and lowest baseline LV ejection fraction presented higher mortality (266). HFrEF patients who present regression of ventricular dilation or increased ejection fraction after treatment have better quality of life. Hoshikawa et al. (101) observed that patients with no reversal of cardiac dilation, after therapy with ACEI, angiotensin II receptor antagonist (ARA) and \beta-blockers, died during the follow-up, which lasted an average of 5 yr. The extent of LV RR at 1 to 6 mo after starting the therapy is predictive of a long-term prognosis (101). An extensive description of RR in HFrEF is presented in Ref. 129.

Less is known regarding the impact of RR on HfPEF mortality and morbidity partly and probably due to population heterogeneity, including variability in the underlying disease and other comorbidities and to unresolved pathophysiological mechanisms during the course of the disease. Nevertheless, exercise training was shown to improve exercise capacity and physical dimensions of quality of life in HfPEF by triggering atrial RR (decreased left atrial volume) and improved LV diastolic function (decreased E/E’ ratio) (58). Despite being a valvular disease, aortic stenosis frequently courses with diastolic dysfunction and presents with features similar to HfPEF. Thus, after aortic valve replacement (AVR), these patients provide a substantial amount of knowledge about the reversibility of LV diastolic dysfunction and the recovery of cardiac structure. AVR results in improvement of overall cardiac pump performance, diastolic function (76, 147, 173, 252), and subendocardial dysfunction earlier than regression of LVH (102, 147). Such a degree of preoperative subendocardial disturbances may represent early changes that if ignored are likely to substantiate and become irreversible. Thus the presence of such abnormalities in symptomatic patients, even with normal ejection fraction, may reinforce the need for AVR to maintain overall integral ventricular function and to avoid potential clinical complications (147). Apart from this study, several studies have shown that the improvement of ventricular performance, assessed namely by LV systolic strain, precedes changes in LVH (109). For instance, Villari et al. (251) showed that after AVR, diastolic perfusion increases due to the reduction of perivascular compression, which improves myocardial blood flow and restores the coronary vasodilatation reserve, decreasing the probability of myocardial ischemia and facilitating molecular mechanisms of RR. Importantly, current clinical evidence does not support regression of LVH as a surrogate marker for (short-term) improvement of HfPEF (97). In another study, including 45 patients with severe aortic stenosis, Poulsen et al. (193) reported 20 and 31% decreases in LV mass index, 19 and 22% decreases in LV-end-diastolic volume, 31 and 31% decreases in LV end-systolic volume, and 6.3 and 6.3% increases in LV ejection fraction, 3 and 12 mo following AVR, respectively. Interestingly, other studies demonstrated that RR occurs mostly within the first 6 mo following AVR since 90% of the changes in cardiac volumes and ejection fraction are complete within this time frame (16) in which 75% of patients display a significant reduction of LV mass. However, a multivariate analysis showed that LV mass remained 5 SDs above normal for more than 85% of the population without an evident explanation based on the age, sex, coronary artery disease, and pre-AVR characteristics such as gradient, valve type, and cross-clamp time (16). However, it is important to have in mind that in the clinical setting of HfPEF patients with compound comorbidities diastolic dysfunction may occur independently of LVH. This may explain why current ap-
approaches to reduce LVH have not been effective in improving symptoms and prognosis in HfP EF (97). Specifically in aortic stenosis, there are several possible explanations for incomplete LV mass regression after AVR: 1) AVR itself does not restore the transvalvular gradient to normal; 2) the afterload is surgically relieved only at the valve level; 3) the surgically induced relief of afterload may be counterbalanced by the resultant increase of another type of afterload, namely arterial hypertension (107); 4) coronary atherosclerosis seems to play a role in RR (16); and 5) there is established myocardial fibrosis.

Regarding myocardial fibrosis, the ECM undergoes a complex process of remodeling wherein collagen deposition holds an important role. In fact, we have shown the importance of fibrosis in a low-risk cohort of patients with severe aortic stenosis. In these patients, higher levels of fibrosis had a negative prognostic impact and represented a predictor of events, independent of other well-established prognostic factors such as ejection fraction, age, baseline LV mass index, or New York Heart Association (NYHA) class (these data are part of a PhD thesis in Ref. 78). Milano et al. (170) in a retrospective analysis demonstrated that the 10-yr survival rate was lower in patients with severe fibrosis, calculated from myocardial biopsies obtained during AVR surgery, and there was no significant improvement in NYHA class (170). In both experimental and clinical observations of LV pressure overload, increased TIMPs levels have been identified, which in turn have been postulated to favor a reduction in ECM turnover, contributing to myocardial fibrosis and LV dysfunction (274). In fact, in aortic stenosis, there is an increased production of collagen and a shift towards inhibition of collagen degradation (63, 64, 98). When compared with controls, myocardial biopsies of aortic stenosis patients have a higher expression of collagens and an upregulation of TIMP-1 and -2 mRNA, favoring inhibition of collagen degradation, which significantly correlates with the degree of fibrosis (98). Therefore, not surprisingly, antifibrotic drugs targeting ECM changes represent an attractive strategy aiming for RR. In this context, animal models of reversible aortic banding have delivered important findings, namely complete regression of MMP and TIMP expression as well as an association between changes in LV mass index and MMP/TIMP ratio after debanding (78). In the same animal model but with a more prolonged period of aortic banding, the fibrotic content remained increased after debanding (19). Moreover, collagen isoform content suffers alterations during RR with a predominance of collagen isoform I early after debanding that shifts to isoform III a few days later (18). In a mouse model of pressure overload, TIMP-4 overexpression was shown to provide beneficial effects for survival and cardiac function and to mute the fibrotic response, since TIMP-4 overexpression may be beneficial in modulating adverse ECM remodeling in the context of pressure overload (274).

The majority of “HfP EF” animal models consist of cardiac pressure-overload models that further develop LVH and diastolic dysfunction. For instance, TAC and systemic hypertension animal models develop LVH accompanied by diastolic dysfunction, ECM, and cardiomyocytes changes. While these observations support a role for LVH in mediating diastolic dysfunction and as a therapeutic target, many of these models later develop HFrEF, impeding translation of these results to the multifactorial setting of clinical HfP EF. For instance, TAC is a well-established surgical technique for inducing LV chronic pressure overload and consequently LVH. Moderate TAC imposed at an early age triggers concentric LVH with compensated chamber performance, markedly with prominent diastolic filling abnormalities. For instance, Yorkshire pigs subjected to surgical banding of the ascending aorta for 5 mo exhibited LVH with increased stiffness and normal systolic function, compared with control pigs (108). However, in severe TAC and long-standing aortic constriction, the myocardial remodeling will, eventually, lead to LV dilation, impairment of systolic function, and progression of the abnormal filling (150). A major limitation of using aortic-banded or hypertensive models is that the majority of patients with HfP EF continue to have HF symptoms even when the blood pressure is controlled. Conversely, less than 50% of HfP EF patients have LVH and often show no evidence of LV dilatation, thus making the value of the current preclinical model questionable (1). Nevertheless, in a variety of HFrEF models (e.g., rodents, rabbit, dogs), interference with LV hypertrophic signaling pathways reliably reduces LVH and improves diastolic function often independently of alterations in blood pressure.

Besides improving function, the beneficial effects of exercise have been shown to be very promising on attenuating or reversing ECM changes during the course of RR (282). For instance, chronic low-intensity interval exercise training attenuated fibrosis and impaired cardiac mitochondrial function (60) and coronary vascular dysfunction (61) in a miniature swine animal model of aortic constriction. Moreover, reduced fibrosis, normal MMP-2 and TIMP-4 expression, and increased collagen III isoform mRNA levels were accompanied by an improvement in diastolic function following chronic training (162).

In HFrEF, LVADs used as a “bridge to recovery” (129) have been shown to be capable of improving heart-rate reserve (125) systolic and diastolic function (95, 176), with no evidence of subsequent regression (54). The improvement of molecular mechanisms such as improvement of β-adrenergic density on ventricular cardiomyocytes (125), enhancement of developed tension and calcium cycling upon catecholamine stimulation (47), upregulation of sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA) (9, 96), or recovery of T-tubule structure and function (105) underlies LVADs benefits for contractile function. Also, in HfReF, cardiac resynchronization, used to counter the delay in electrical conduction that leads to desynchronous contraction in HF (129), was implicated in the reduction of LV volumes after a period of several months and this adaptation persisted even if pacing was temporarily suspended (275).

The RAAS seems to be a key factor in ECM remodeling process. Mechanical stretch induces local production of angiotensin II, which in turn stimulates the release of multiple growth factors and cytokines from cardiac fibroblasts that act in an autocrine and paracrine fashion, affecting the progression of hypertrophy, remodeling (206, 258), and RR (232). HFrEF patients treated either with ACEI or with a combination of ACEI and ARA show improvements in mortality rate and in disease progression partly due to capacity to reduce ventricular mass (55, 86, 127, 189). Besides RAAS, other pharmacological interventions, such as β-blockers, have been shown to impact myocardial hypertrophy. For example, compared with placebo treatment, carvedilol decreased LV mass while improving
cardiac geometry in patients with HFrEF after 4 mo of treatment (156). Moreover, HFrEF patients treated with LVAD in combination with ACEI medication presented a decrease in myocardial collagen content and myocardial stiffness compared with patients with LVAD therapy alone (126), which can be potentially relevant for HFrEF. Disappointingly, despite its actions on hypertrophy and ECM, these studies failed to improve the clinical outcome of HFrEF patients. Thus more studies are necessary to better understand the role of this collagen shift in RR mechanisms, particularly in HFrEF.

Altered Signaling Pathways in HFpEF as Potential Targets for Reverse Remodeling

While myocardial remodeling and RR have been extensively studied for HFrEF, the current knowledge regarding detailed regulatory mechanisms of HFpEF pathophysiology is scarce. Consequently, despite several clinical trials attempting novel treatments for HFpEF, none have delivered convincing results. Thus it is mandatory to further explore and understand the most altered signaling pathways underlying it. This section will cover some of the most promising signaling pathways aiming to rewind myocardial remodeling in HFpEF patients including calcium handling and myofilamentary proteins alterations, ECM changes, activation of cyclic guanosine monophosphate (cGMP) signaling cascade, inflammation, and oxidative stress.

Calcium-handling proteins. Cardiomyocyte contractile function is controlled by Ca$^{2+}$-dependent myofilament activation and relaxation as well as by passive visco-elastic properties largely determined by the myofilaments (e.g., titin). The relationship between “systolic” and “diastolic” function at the cellular level is expected to be highly interdependent. As in the whole organ, mechanical energy stored in the sarcomeric protein titin during contraction contributes to recoil during relaxation. On the other hand, resting cardiomyocyte tension in diastole is a determinant of contractile force during systole (97). Normally, contraction starts with depolarization of the cell membrane, which triggers the entry of small quantities of Ca$^{2+}$ into the cardiomyocyte stimulating the nearby ryanodine receptors (RyR) (15). This results in a large increase in intracellular Ca$^{2+}$, which acts on troponin C (TnC) to activate cross bridges between actin and myosin filaments in the sarcomeres, causing cardiac contraction (183).

Cardiomyocyte relaxation starts as soon as membrane L-type Ca$^{2+}$ channels close, the extrusion of Ca$^{2+}$ from the cytosol by the Na$^{+}$/Ca$^{2+}$ exchanger starts, and sequestration of Ca$^{2+}$ by SERCA to the sarcoplasmic reticulum (SR) begins. The action of SERCA is controlled by phospholamban. When it is dephosphorylated, phospholamban inhibits SERCA, and when it is phosphorylated, this inhibition is lost, SERCA is activated, the cytoplasmic Ca$^{2+}$ decreases, and relaxation occurs (15).

Several components in the Ca$^{2+}$ cycling process could be disturbed and significantly impact diastolic and systolic function (Fig. 2). In HFrEF there is evidence supporting a decrease in intracellular Ca$^{2+}$ transient and diminished SR Ca$^{2+}$ content, an outcome that constitutes the major origin of the altered contractility. This can be attributed to alterations in the expression/activity of different Ca$^{2+}$ regulatory proteins, in particular a decrease in SERCA levels observed in experimental and human HFrEF (167). Modifications in RyR are also present in
HFrEF. For instance, hyperphosphorylation of RyR by Ca$^{2+}$/calmodulin-dependent protein kinase II (CaMK-II) or protein kinase A (PKA) might cause a diastolic leak of Ca$^{2+}$, lowering the Ca$^{2+}$ content of the SR, thereby reducing the quantity of Ca$^{2+}$ released during the subsequent activation. This weakens systolic contraction and, by raising cytoplasmic Ca$^{2+}$ during diastole, it also interferes with myocardial relaxation (27). New therapeutic strategies for HFrEF are underway to modify the actions of Ca$^{2+}$, including enhancing the sensitivity of cardiac myosin to Ca$^{2+}$ (159), repairing the leak in RyR channels, and increasing expression of SERCA (161). Contrarily to HFrEF, where cardiomyocyte contractile dysfunction is predominant, in HFpEF cardiomyocytes stiffness and relaxation dysfunction become more relevant and can be also associated with Ca$^{2+}$ overload induced by higher sensitivity of myofilaments to Ca$^{2+}$ or decreased rate of Ca$^{2+}$ reuptake via SERCA and alterations in Na$^+$/Ca$^{2+}$ exchanger. Changes in the phosphorylation state of proteins that modify SERCA activity, such as phospholamban, CaMK-II, and calsequestrin, have been associated with increased levels of cytosolic diastolic Ca$^{2+}$, inducing diastolic dysfunction associated with impaired active relaxation and/or increased passive stiffness of cardiomyocytes (279). Multiple approaches have been utilized to alter the function of phospholamban, SERCA, and the Na$^+$/Ca$^{2+}$ exchanger with the goal of improving Ca$^{2+}$ handling and thus diastolic function, including drugs that mimic, inactive, or decrease phospholamban and adenoviral gene delivery of SERCA, to increase its activity (6). The use of the Na$^+$/Ca$^{2+}$ exchanger inhibitors was also explored in Wistar rats subjected to subtotal nephrectomy (acute treatment with SEA0400), showing a normalization of cytosolic Ca$^{2+}$ transients, an improvement of trans-sarcomemal Ca$^{2+}$ export, and a decrease in SR Ca$^{2+}$ leak in Na$^+$/Ca$^{2+}$ exchanger, in line with a role for reverse mode Na$^+$/Ca$^{2+}$ exchanger activity in HFpEF. This cellular changes were accompanied by in vivo enhancement of LV active relaxation as shown by a decreased isovolumetric relaxation constant (194).

Ranolazine, a new anti-ischemic and anti-arrhythmic medication, inhibits the late influx Na$^+$ current (typically increased in HF) leading to decreases in Na$^+$ accumulation. Consequently, Ca$^{2+}$ extrusion through the Na$^+$/Ca$^{2+}$ exchanger will increase and thereby diastolic tension decreases and relaxation improves (220). In fact, administration of ranolazine in DOCA-salt rats improved diastolic function through modulation of myofilament activity, including Ca$^{2+}$ response and cross-bridge kinetics (155).

Parvalbumin is a Ca$^{2+}$ buffer protein expressed in the fast-twitch skeletal muscle and not normally expressed in the heart. In the former parvalbumin facilitates rapid relaxation by buffering Ca$^{2+}$ away from myofilaments after contraction. The therapeutic potential of parvalbumin has been tested for increasing the relaxation rate of the heart under diastolic dysfunction conditions. In fact, gene transfer of parvalbumin to the heart triggered an in vivo improvement in different relaxation parameters in rats with slowed cardiac muscle relaxation (228) including aged rats (203). Changes in myofilament Ca$^{2+}$ sensitivity, through changes in Ca$^{2+}$ interaction with thin myofilaments (263), such as protein kinase C (PKC)-induced phosphorylation of troponin I (TnI) (104), can also change cardiomyocyte relaxation (104).

CARDIAC MYOSIN-BINDING PROTEIN-C. Cardiac myosin-binding protein-C (cMyBP-C) is a component of the thick filament in cardiomyocytes that modulates the cross-bridge attachment/detachment cycling process (236). Experimental studies have suggested a potential role for cMyBP-C in diastolic function. For instance, the cMyBP-C null mouse model (116) and cMyBP-C homozygous and heterozygous knockin mouse exhibited diastolic dysfunction with elevated E/E’ (120). Moreover, mutations in this protein were observed in patients with hypertrophic cardiomyopathy, among whom a significant percentage presented diastolic dysfunction, demonstrated by slowed cardiac relaxation (225). Apparently, the phosphorylation of cMyBP-C is able to modulate diastolic function (10, 41, 222, 237) (Fig. 3) by enhancing cardiac lusitropy whereas the absence of phosphorylation depresses lusitropy (226). cMyBP-C can be phosphorylated by different kinases known to be altered in HFpEF, including PKA (77), PKC (270), and CaMK-II (202). However, the development of therapies to increase or maintain cMyBP-C phosphorylation in HFpEF is still challenging, since in HF different signaling pathways can lead to alterations in cMyBP-C phosphorylation status. For instance, β-adrenergic receptor activation triggers PKA phosphorylation of cMyBP-C and can also activate G-protein receptor kinase-2 signaling (198), a pathway that can cause maladaptive remodeling (2). Additionally, in a hypertensive animal model, diastolic dysfunction was accompanied by a decrease in S-glutathionylation of cMyBP-C, depression in myofilament cross-bridge kinetics (155), cardiac tetrahydrobiopterin (BH4) depletion, and nitric oxide (NO) synthase (NOS) dysfunction (216). Moreover, cMyBP-C glutathionylation correlated with the presence of diastolic dysfunction (112). Feeding hypertensive mice with BH4 increased cardiac stores of this molecule and improved diastolic dysfunction. The authors suggested that by depressing S-glutathionylation of cMyBP-C, BH4 ameliorates diastolic dysfunction by reversing a decrease in cross-bridge turnover kinetics. Importantly, preliminary studies from the same authors found that modified cMyBP-C can be measured in blood and is elevated in patients with diastolic dysfunction (112).

TITIN. Titin, the largest human protein spanning the Z line to the M line of the sarcomere, has an important role in diastolic function. Titin, along with collagen, is the one of the major determinants of myocardial stiffness (144). As the SL increases, the contribution of titin decreases and collagen becomes mainly responsible for myocardial stiffness (>2.4 μm) (94). In adult cardiomyocytes, titin is expressed in two isoforms, the stiff N2B and the compliant N2BA. Stiffness of cardiomyocytes is defined by the ratio between these isoforms, for example, an increase in the N2B/N2BA ratio is associated with higher stiffness (268). Recently, Schwarzl et al. (207) observed myocardite hypertrophy, titin isoform shift, toward the stiffer titin isoform N2B, and reduced total titin phosphorylation in LV biopsies from a large animal model of hypertension and hyperlipidaemia. Posttranslational modifications are able to modify cardiomyocyte stiffness (20) as certain titin domains are substrates for different kinases (Fig. 3). While titin phosphorylation by PKA (134, 272), protein kinase G (PKG) (263), CaMK-II (93), and extracellular signal-regulated kinase 2 (ERK-2) (195) decrease cardiomyocytes passive tension (134, 272), PKC triggers an increase in passive tension (99). Specifically, in HFpEF, low myocardial PKG activity was associ-
ated with raised passive tension and with increased oxidative stress (244). For instance, a study using a robust animal model of HFpEF, the obese ZSF1 rats (hypertensive and diabetic), demonstrated that hypophosphorylation of titin contributed to the underlying myocardial diastolic dysfunction observed in these animals (92). Calcium is also responsible for cardiomyocyte stiffness alterations (20), namely, by its binding to the titin PEVK region, which increases stiffness (213), or by the activation of calcium-binding protein 1 (S100A1), a protein that directly regulates PEVK-actin interactions in the sarcomere (169). During systole (elevated Ca$^{2+}$ levels), the complex Ca$^{2+}$-S100A1 triggers the inhibition of PEVK-actin interaction, which would diminish PEVK-actin-dependent motility inhibition, facilitating myocardial contraction (169). At lower Ca$^{2+}$ levels, this mechanism is reduced and titin-actin interactions lead to increased passive tension. It can be hypothesized that decreased levels of S100A1, observed in HF, can promote an increase in the titin-actin interaction, which contributes to the increased myocardial stiffness (56) observed in a great proportion of HFpEF patients. In fact, S100A1 overexpression improves cardiac function parameters (29, 201) and reverses LV remodeling in an animal model of myocardial infarction (192). However, it is still necessary to understand if S100A1 expression provides beneficial effects for HFpEF conditions. Furthermore, oxidative formation of disulphide bonds in titin’s N2B region leads to increased cardiomyocyte stiffness (87) and S-glutathionylation of Ig domain cysteins is associated with a decrease in titin stiffness (3). Since higher passive tension of cardiomyocytes, as a consequence of titin changes, underlies the diastolic dysfunction of HFpEF, we think that targeting posttranslational modification on titin as herein described detain an enormous potential to reverse diastolic dysfunction in this condition.

Extracellular matrix. The activation of myofibroblasts is the key of fibrotic tissue remodeling. As already mentioned, LVH is caused by an abnormal accumulation of collagen and other ECM components in the extracellular space. This reactive and progressive interstitial fibrosis contributes to myocardial stiffness and, ultimately, ventricular diastolic dysfunction, and it is believed to result from the persistent activation of cardiac myofibroblasts. Several studies have demonstrated that circulating hormones such as ET-1 and angiotensin II and fibrogenic cytokines/proteins such as TGF-$\beta$ act in a network that contributes to myofibroblast differentiation and persistence (140). In fact, the majority of experimental antifibrotic strategies attempt to target activation, proliferation, and/or recruitment of fibroblasts (269).

TGF-$\beta$ is related to ECM gene expression and promotion of ECM deposition by simultaneously suppressing and inducing MMP and TIMP gene expression, respectively. Angiotensin II levels are increased in pressure overloaded hearts and are associated with remarkable profibrotic proprieties, in part, through stimulation of TGF-$\beta$ (32, 205). Aldosterone also mediates vascular and cardiac remodeling and binds to the mineralocorticoid receptor stimulating cardiac fibroblasts and increasing collagen synthesis and deposition (259). Blocking the RAAS with ACEI and ARA was shown to effectively reduce fibrosis in different animal models (164, 235) and also in HFpEF patients (215). It is expected that these targets will also provide beneficial effects for HFpEF, since myocardial fibrosis is associated with the appearance of diastolic dysfunction in these patients. In an animal model of pressure overload,
inhibition of TGF-β, with neutralizing antibodies, attenuated cardiac fibrosis and improved diastolic function without affecting cardiomyocyte hypertrophy (136). For instance, increased cardiac expression of monocyte chemoattractant protein (MCP-1) preceded TGF-β1 upregulation and was associated with cardiac fibrosis and diastolic dysfunction (136). The chronic treatment with an anti-MCP-1 antibody attenuated myocardial fibrosis and ameliorated diastolic dysfunction of hypertensive Wistar rats, without affecting blood pressure and systolic function (137), thus representing a potential new strategy to prevent inflammation and the consequent myocardial fibrosis and diastolic dysfunction.

Advanced glycation end-products (AGEs), formed when glucose interacts nonenzymatically with proteins, can cause increased stiffness of the ECM directly by cross linking collagen or elastin and indirectly by stimulating the production of collagen and depleting NO thereby increasing oxidative stress (23). The beneficial effects of AGEs breakers in ventricular distensibility and arterial compliance improvement has been studied in recent years. In fact, elderly patients with HFP EF treated for 16 wk with an AGEs breaker showed LVH regression and diastolic function improvement (149).

Activation of cGMP signaling cascade. cGMP-dependent protein kinase, PKG, is a serine/threonine kinase presenting three isoforms, PKG1α, PKG1β, and PKG2 (70). PKG1α is the primary cardiac isoform (229) and, in the cardiovascular system, it phosphorylates several key transcription factors and sarcomeric proteins involved in hypertrophy signaling, diastolic relaxation, myocardial stiffness, and vasorelaxation (62, 265). PKG is activated upstream by increased levels of cGMP. Interestingly, decreased PKG activity related to low concentration of cGMP was found in LV biopsies from HFP EF patients (244) and was associated with higher cardiomyocytes passive tension and greater myocardial oxidative stress (244). Excitingly, PKG administration decreased cardiomyocytes passive tension, which could be explained, as previously mentioned, by titin phosphorylation by PKG (244). This suggests that increases in cGMP and consequently activation of PKG could represent a potential therapeutic target for HFP EF. Elevation of myocyte cGMP levels in myocytes can be target by the natriuretic peptides [atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP)], NO, or sildenafil that phar- 

Antihypertrophic effects in the wild-type and not in the Lzm mouse subjected to TAC (21). Subsequent studies have demonstrated that phosphorylation of receptor potential canonical channel 6 (TRPC6) via PKG suppresses Ca2+ current into the cell. Low cytosolic Ca2+ prevents activation of calcineurin, which, in turn, decreases the desphosphorylation levels of nuclear factor of activated T-cells (NFAT), avoiding its nuclear translocation and the consequent expression of prohypertrophic genes (128, 172).

Preclinical studies suggest that inhibition of PDE5A reverses cardiac structural and functional remodeling and enhances vascular, neuroendocrine, and renal function. However, despite showing preclinical promise, sildenafil was not successful in clinical trials for HFP EF (196). Recently, Lee et al. showed that phosphodiesterase type 9A (PDE9A) expression was increased in the myocardium of patients with various forms of HF, especially HFP EF (141). Using different approaches, the authors showed that PDE9A is localized in different compartments of the sarcomere in myocytes and that PDE5A and PDE9A target cGMP in the NO- and ANP-signaling pathways, respectively. Moreover, the genetic or selective pharmacological (PF-9613) inhibition of PDE9A protected the myocardium against neurohormones actions and sustained pressure-overload stress. According to Bray (28), this study showed that PDE9A, unlike PDE5A, specifically hydrolyzes NO-independent, ANP-coupled cGMP. Therefore, selective PDE9A inhibitors, such as PF-9613, could have greater effectiveness than PDE5A inhibitors for treating cases of HFP EF in which NO production is compromised (28).

Inflammation and oxidative stress. As already mentioned above, noncardiac comorbidities are highly prevalent in HFP EF, and they have the capacity to induce a systemic inflammatory state. In fact, clinical studies with HFP EF patients, described high circulating levels of inflammatory markers such as interleukin 6 (IL-6) and TNF-α (33, 39, 114), soluble ST2 (211), and plasma pentraxin 3 (166). Pentraxin 3 levels were described high circulating levels of inflammatory markers such as interleukin 6 (IL-6) and TNF-α (33, 39, 114), soluble ST2 (211), and plasma pentraxin 3 (166). Pentraxin 3 levels were recently correlated with the risk of cardiovascular events in HFP EF patients (165). The systemic inflammatory state induced by HFP EF comorbidities triggers the production of ROS, which can interfere with kinase expression/activity (83) and with NO-related signaling in different ways. In fact, NOS uncoupling triggered by oxidation has been associated with pathologic concentric remodeling and diastolic dysfunction (231) and with endothelial dysfunction (139). When NOS uncoupling occurs, a superoxide molecule is produced, inducing oxidative stress (199). Besides NOS alterations, higher levels of ROS lead to oxidation of the NO receptor soluble guanylate cyclase (sGC) resulting in impaired NO-induced cGMP production (239) and thus in low PKG activity. Increased inflammation also triggered activation of TGF-β, resulting in transdifferentiation of fibroblasts into myofibro- blasts, which produce more collagen, contributing to diastolic dysfunction in HFP EF patients (262). Paulus and Tschope (185) proposed a novel paradigm for HFP EF, in which comorbidities contribute to a systemic inflammatory state, which induces oxidative stress in the coronary microvascular endothelium. In turn, ROS leads to formation of peroxynitrite (ONOO-) and reduced NO bioavailability, both of which lower sGC activity in adjacent cardiomyocytes. The consequent decrease of PKG activity increases resting tension of cardiomyocytes due to titin hypophosphorylation and removes...
the brake on prohypertrophic stimuli, promoting cardiomyocyte growth. Moreover, expression of both vascular cell adhesion molecule (VCAM) and E-selectin by endothelial cells favors migration of monocytes into the subendothelium, resulting in increased levels of TGF-β release and myocardial deposition of collagen (185, 269). Recently, Franssen et al. (71) provided comprehensive evidence for microvascular endothelial activation, high oxidative stress, endothelial nitric oxide synthase (eNOS) uncoupling, and low NO levels in the LV myocardium of HfPfEF patients. These findings were reproduced in obese ZSF1 rats, which develop HfPfEF at 20 wk of age, in contrast to lean ZSF1 rats (hypertensive controls), which maintain normal LV function after a similar time period. They also demonstrated that low myocardial NO level was associated with reduced myocardial cGMP/PKG signaling in obese ZSF1 rats (71).

Therapeutic approaches using antioxidants have been shown to improve cardiovascular function in experimental models, but clinical trials have repeatedly failed to reproduce such effects (81). For example, mouse overexpressing catalase targeted to mitochondria have an ~20% extension of lifespan, a reduction of age-induced hypertrophy and diastolic dysfunction, and a concomitant attenuation of age-dependent increases in cardiac mitochondrial DNA deletions and oxidative damage (45, 204). These animals were also protected from angiotensin II-induced cardiac hypertrophy, Gqαq overexpression-induced HF, and attenuated TAC-induced phenotype HF, including protection of cardiac mitochondrial proteome and metabolic remodeling, attenuation of mitochondrial unfolded protein response, and apoptotic pathway activation (43). In contrast, overexpression of wild-type catalase targeted to peroxisomes did not provide any improvement in the response to angiotensin II or Gqαq overexpression (44).

"Old" and "New" Promising Therapeutic Targets to Promote Reverse Remodeling in HfPfEF

Therapy in HF is aimed at amelioration of symptoms, improvement in function/quality of life, and/or prolongation of life. In general, HF therapies associated with positive long-term clinical outcome, such as reduced hospitalizations, mortality, or both, have been intimately associated with beneficial RR (126). One of the challenges and barriers in treating HF is the heterogeneity of the clinical syndrome. In patients with chronic HfRfEF, both the survival and quality of life have improved with the use of β-blockers, RAAS inhibitors, and with devices, including pacemakers, which enhance cardiac synchronization, and implanted cardiac defibrillators (27). Many of these pharmacological approaches successful for HfRfEF have been attempted for HfPfEF. Such is the case of the RAAS and β-adrenergic signaling pathways, which seemed appealing due to their link to fibrosis, hypertension, and fluid balance. However, results from different clinical trials failed to provide consistent conclusions about the beneficial effects of HfPfEF standard therapies such as β-blockers, ACEI, ARA, aldosterone antagonists, PDE5A inhibitors, statins, and calcium channels blockers on HfPfEF. The most important pharmacological approaches already attempted for HfPfEF in clinical trials are summarized in Fig. 4.

Another drug with animal-tested benefits is ranolazine, which is a novel anti-ischemic and anti-arrhythmic medication used clinically to treat angina without lowering blood pressure or heart rate (37). The Ranolazine for the Treatment of Diastolic Heart Failure (RALI-DHF) study revealed that 30 min of ranolazine infusion improved hemodynamics, including LV end-diastolic pressure and pulmonary capillary wedge pressure. However, relaxation parameters, namely time constant (tau) and the rate of decline of LV pressure per minute, were unaltered. Also, the ratio E/E′ did not change 2 h after infusion (158). As suggested by the authors their findings are consistent with an in vitro study that showed lack of an improvement by ranolazine in active relaxation of cardiomyocytes from the failing heart, although diastolic dysfunction over time was improved (220). Mechanistically, in HfPfEF two types of relaxation exist, the passive (or late-phase) relaxation, which may well be related to diastolic dysfunction and hence improves with ranolazine due to improvement in slow Na+/Ca2+ dependent Ca2+ overload, and active (or early) relaxation, which is more related to SR reuptake of Ca2+ from beat to beat. In fact, SR Ca2+ content did not change significantly in the presence of ranolazine (220). Nevertheless, larger clinical trials are needed to better evaluate the potential role of ranolazine in HfPfEF.

In a placebo-controlled phase II study, Ivabradine, a selective inward funny (If) channel inhibitor, which reduces heart rate without a negative inotropic effect, significantly improved aerobic exercise capacity (VO2 peak), ventilatory efficiency (VE/VCO2), myocardial relaxation (E′), and diastolic cardiac reserve in patients with HfPfEF, independent of the maximal heart rate response to exercise (131).

In a small pilot cross-over trial of 12 HfPfEF patients, anakinra, an interleukin 1 (IL-1) blocker, led to a significant reduction in C reactive protein, a marker of systemic inflammation, and an improvement in aerobic exercise capacity (VO2 peak) and ventilatory efficiency (oxygen uptake efficiency slope) (247).

As already mentioned, one of the mechanisms behind HfPfEF is deregulation of the NO-cGMP protein kinase pathway. Two new drugs are currently under investigation to test whether this pathway can be significantly improved by either the angiotensin II receptor-neprilysin inhibitor LCZ696, which has been demonstrated to reduce NT-proBNP in a phase II trial in 300 patients with HfPfEF (217), or by the sGC stimulator vericiguat, which is able to increase cGMP (190).

![Fig. 4. Effects of standard heart failure-therapies, such as β-blockers, angiotensin II-converting enzyme inhibitor (ACEI), angiotensin II receptor antagonist (ARA), and phosphodiesterase type 5A (PDE5A) inhibitors on HfPfEF. Numbers in parentheses in A–C refer to reference numbers. A: studies of β-blockers effects in long-term outcome (mortality) and/or quality of life (exercise capacity and HF hospitalizations). B: studies of ACEI and ARA effects in long-term outcome (mortality) and/or quality of life (exercise capacity and HF hospitalizations) of HfPfEF patients. C: preclinical studies of the PDE5A inhibitor sildenafil effects in HfPfEF. Moreover, observational studies suggest that statin therapy is associated with lower mortality in patients who have diastolic HF (73). This was confirmed in a large-scale cohort of patients with HfPfEF (CHART-2 study) (180). Ivabradine, an inward funny (If) channel blocker, also had a positive impact in exercise capacity of HfPfEF patients (197).](http://ajpheart.physiology.org/)

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A  Efficacy of β-blockers in HFpEF

- Carvedilol, metoprolol succinate and bisoprolol (OPTIMIZE-HF) (68)
- Nebivolol (ELANDD) (40)
- Carvedilol (J-DHF and SWEDIC) (271, 13)
- Nebivolol (SENIORS) (248)

No effects on long-term outcome and/or quality of life

Effects on long-term outcome and/or quality of life

Different observational studies using β-blockers (5, 112, 49, 181)

Meta analysis studies (151, 11)

Ongoing study: Beta-PRESERVE (278)

B  Efficacy of ACEIs, ARAs and aldosterone antagonists in HFpEF

- Spironolactone (TOPCAT study) (191)
- Irbesartan (I-PRESERVE study) (163)
- Candesartan (CHARM-PRESERVED study) (276)
- Perindopril (PEP-CHF study) (35)

No effects on long-term outcome and/or quality of life

- Impact of ACEI and ARA (OPTIMIZE-HF study) (68)
- LCZ696 (PARAMOUNT study) (217)
- Sperilactone (ALSO-HF study) (59)

Effects on long-term outcome and/or quality of life

- Improved left ventricular diastolic function but did not affect maximal exercise capacity, patient symptoms, or quality of life
- Impact of different ACEi therapies in patients with DHF (238)

Ongoing study: PARAGON-HF(65)

C  Efficacy of PDE5A inhibitors in HFpEF

- Impact of Sildenafil in HFpEF associated pulmonary hypertension:
  - Without pulmonary artery pressure reduction and improvements in invasive haemodynamic or clinical parameters (100)
- Sildenafil (RELAX study):
  - Without improvement in exercise capacity or clinical outcomes (196)

Negative effects

Positive effects

- Impact of Sildenafil in HFpEF associated pulmonary hypertension:
  - Improvements in pulmonary pressure and vasomotility, right ventricle function and dimension, left ventricular relaxation and distensibility (88)

Controversial study (69, 11, 84)
Alagebrum chloride (ALT-711) has been tested as a AGEs cross-link breaker and may improve ventricular distensibility and arterial compliance. A prospective, open-label trial of alagebrum in elderly patients found that in clinically stable HFP EF the treatment with ALT-711 caused regression of LVH, improved Doppler indexes of diastolic function, and enhanced quality of life without altering blood pressure, arterial stiffness, or exercise tolerance (149). Prevention of the formation of new AGEs with exercise and breakdown of already formed AGEs with ALT-711 may represent a therapeutic strategy for age-related ventricular and vascular stiffness (223). Other treatments appear promising in preclinical studies but await translation (7, 130).

The beneficial effects of lifestyle changes in HFP EF are also being explored. Currently, the most evidenced-based promising strategy to improve exercise intolerance in HFP EF patients appears to be exercise training, but the optimal approach is still unknown (241). Sixteen weeks of exercise training increased peak VO2, ventilatory anaerobic threshold, 6-min walk distance, and physical quality-of-life scores in patients with HFP EF (124). A recent trial showed an improvement in diastolic function, with reduction of the E/E’ ratio, in HFP EF group patients subjected to aerobic interval training, compared with general health care groups (72). These studies suggest an important role for exercise in HFP EF patient’s life, which must be considered in the treatment of this syndrome. Yet, it is still necessary to think about how to effectively and safely implement exercise training in an aged and frail population, such as HFP EF patients (241). Regarding this, exercise training may pair particularly well with other nonpharmacological interventions, including lifestyle interventions, such as nutrition, and disease management strategies. Indeed, clinical cardiac rehabilitation programs for coronary artery disease patients routinely include these in a multidimensional approach. In this light, research studies that strive to isolate the effects of exercise training alone likely underestimate the full range of potential benefits from a rehabilitation approach to HFP EF (123).

Conclusion

HFP EF is a major and growing public health problem. To date, no treatments have convincingly improved its clinical outcomes nor have positively altered RR. Moreover, the pathophysiological mechanisms underlying this syndrome remain to be clarified partly due to 1) the presence of highly confounding comorbidities that frequently coexist in HFP EF population; 2) limited myocardial biopsies from HFP EF patients; 3) the lack of proper animal models mimicking all the pathophysiological features of the human disease; and, lastly, 4) the long list of failed therapeutic strategies tested. Nevertheless, new proteins, protein-protein interactions, and signaling pathways are being explored as potential new targets for preventing HFP EF or promoting its RR.

REFERENCES


180. Nair N, Kumar S, Gongora E, Gupta S.


Yamasaki R, Wu Y, McNabb M, Greaser M, Labeit S, Granzier H. Protein kinase A phosphorylates titin’s cardiac-specific N2B domain and
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