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Left ventricular dysfunction in heart failure with preserved ejection fraction—molecular mechanisms and impact on right ventricular function

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Abstract: The current classification of heart failure (HF) based on left ventricular (LV) ejection fraction (EF) identifies a large group of patients with preserved ejection fraction (HFpEF) with significant morbidity and mortality but without prognostic benefit from current HF therapy. Co-morbidities and conditions such as arterial hypertension, diabetes mellitus, chronic kidney disease, adiposity and aging shape the clinical phenotype and contribute to mortality. LV diastolic dysfunction and LV structural remodeling are hallmarks of HFpEF, and are linked to remodeling of the cardiomyocyte and extracellular matrix. Pulmonary hypertension (PH) and right ventricular dysfunction (RVD) are particularly common in HFpEF, and mortality is up to 10-fold higher in HFpEF patients with *vs.* without RV dysfunction. Here, we review alterations in cardiomyocyte function (i.e., ion homeostasis, sarcomere function and cellular metabolism) associated with diastolic dysfunction and summarize the main underlying cellular pathways. The contribution and interaction of systemic and regional upstream signaling such as chronic inflammation, neurohumoral activation, and NO-cGMP-related pathways are outlined in detail, and their diagnostic and therapeutic potential is discussed in the context of preclinical and clinical studies. In addition, we summarize prevalence and pathomechanisms of RV dysfunction in the context of HFpEF and discuss mechanisms connecting LV and RV dysfunction in HFpEF. Dissecting the molecular mechanisms of LV and RV dysfunction in HFpEF may provide a basis for an improved classification of HFpEF and for therapeutic approaches tailored to the molecular phenotype.

Keywords: Heart failure (HF); cardiomyocytes; metabolism; calcium; sodium; right ventricle; pulmonary hypertension (PH)

Heart failure with preserved ejection fraction (HFpEF)—clinical and cellular characteristics

Introduction

A 71 years old mildly overweight woman is transferred to the hospital with worsening dyspnea on exertion, unproductive cough and mild ankle edema for a few weeks. Once the diagnosis of heart failure (HF) is established, she faces a 1 in 6 chance to die within 2 years despite optimal current therapy (1). HF is the leading cause for hospital admissions, and more than 26 million people worldwide are affected (2). HF is diagnosed in a large variety of patients with different medical backgrounds. Currently, diagnosis is based on clinical signs and symptoms, and imaging (e.g., echocardiography). HF patients are classified based on left ventricular (LV) ejection fraction (EF) as patients with preserved (HFpEF, LV EF ≥50%), reduced (HFrEF, LV EF <40%) or mid-range EF as basis for clinical management. Inhibition of neurohumoral activation with ACE-inhibitors, angiotensin-receptor blockers or betablockers, and enhancing the availability of natriuretic peptides (NP) with neprilysin inhibitors (sacubitril, in combination with valsartan) are effective treatments in HFrEF but failed to improve prognosis in HFpEF (3-5) (see also "neurohumoral activation" section for discussion). It is commonly argued that HFpEF represents a more heterogeneous disease as suggested by (I) the variety of clinical disease conditions associated with HFpEF (6), (II) very different long-term prognosis if HFpEF patients are classified based on the severity of their predominant clinical features (7), and (III) identification of subgroups of HFpEF patients that respond to medical HF therapy in pooled analyses from randomized controlled trials (RCTs) and observational trials (5,8). Some but not all studies suggest an increased number of co-morbidities in HFpEF vs. HFrEF (9,10). HFpEF patients are older and more likely female (11). Renal failure seems equally common in all HF patients, however, other so-called "non-cardiac" co-morbidities such as chronic obstructive pulmonary disease (COPD), diabetes, obesity and arterial hypertension are more prevalent in HFpEF (10,11).

The cardiac phenotype of HFpEF is characterized by increased LV filling pressure, a reduced LV stroke volume (SV) (at rest or during exercise) and blunted chronotropic response in some patients. Cardiac output (CO) greatly depends on myocardial pump function as well as the elastic properties of the subsequent arterial vasculature and venous blood flow returning to the heart. Their contribution may be of particular importance for right heart function in HF as discussed below (see "RVD in HFpEF"). Ventricular aortic coupling is reviewed in detail by Bernardo et al. in this issue of the Journal. Here, we will focus on molecular and cellular mechanisms of HFpEF at the level of the myocardium.

In the canonical view of HF(rEF) pathophysiology, neurohumoral activation and maladaptive cardiac remodeling are triggered by a reduction in myocardial contractile force that results from the replacement of functional cardiomyocytes by fibrotic scar tissue [e.g., following necrosis, apoptosis or both (12-15)]. While fibrosis is also a hallmark of remodeling in HFpEF (see "fibrosis/extracellular matrix" section), excessive cardiomyocyte death is not typical (16), even though an increased rate of apoptosis is observed in specific cardiomyopathies (17,18). Disease progression in HF is paralleled by an increased mechanical load on the cardiac myocytes, either by increased preload despite reduced SV or by increased afterload with the progression of co-morbidities. Cardiac remodeling in HF is almost invariably associated with cardiomyocyte hypertrophy, which may be even more pronounced in HFpEF vs. HFrEF (19). In HFpEF, cardiomyocytes contribute to diastolic dysfunction by increased passive stiffness (see "the role of titin in impaired cardiomyocyte relaxation" and "inflammatory-driven pathogenic pathways in HFpEF" sections). However, recent evidence indicates that systemic inflammation and metabolic dysregulation also impair excitation-contraction coupling (ECC) and thus active cardiomyocyte relaxation as discussed in "active cardiomyocyte relaxation" section. Hypertrophic cardiomyopathy (HCM), a genetic disorder of the myofilaments, is often associated with diastolic dysfunction, and—even in the absence of outflow tract obstruction—may lead to HFpEF (20). While implications of alterations in myofilament properties are discussed in "myofilament properties" section, we refer to more detailed recent reviews on the pathophysiology and presentation of HCM (20-22).

Increased myocardial passive stiffness

Increased myocardial passive stiffness is a hallmark feature of HFpEF. Two major components are relevant for increased passive stiffness: altered extracellular matrix composition and impaired cardiomyocyte relaxation.

Fibrosis/extracellular matrix

As extensively reviewed before (13,23-25), the amount of collagen, the abundance of collagen type 1 and collagen

cross-linking contribute to increased myocardial stiffness and diastolic dysfunction. In MRI studies, impaired diastolic function correlated with myocardial fibrosis (26), which is predominantly localized perivascularly and diffusely in the interstitial space (26,27) and is considered a hallmark feature of HFpEF (28). Fibrosis has also been related to inflammatory triggers (29). Consistently, myocardial inflammation has also been linked to increased collagen type 1 and enhanced collagen cross-linking in biopsies from HFpEF patients (30). Extracellular matrix is also influenced by other proteins that accumulate in the setting of comorbidities frequently associated with HFpEF. For instance, advanced glycosylation end-products (AGEs) are found in particular in patients with diabetes (31) and have been associated with myocardial inflam¬mation and HFpEF (32), although their role for impaired relaxation is debated (31). Interestingly, extracellular matrix stiffness has been shown to directly affect cardiomyocyte function and Ca^{2+} transients (CaTs) in a HFpEF model (33).

The role of titin in impaired cardiomyocyte relaxation

Titin exists in two main isoforms that contribute to myocardial stiffness, the N2B and the N2Ba isoform springs. As these isoforms show different compliances titin-isoform switch, i.e., an altered N2B/N2BA ratio, is a well understood mechanism contributing to increased diastolic stiffness in a variety of cardiomyopathies (34). It is thus not surprising that isoform switch was identified as a main driver of HFpEF development (7). In addition, titin compliance is influenced by its phosphorylation state, tightly related to local inflammation and oxidative stress (35). It has been suggested that titin and cardiomyocyte related alterations are more important during early as compared to late stages of HFpEF (36). Interestingly, arterial hypertension with LV hypertrophy but without HF was not associated with alterations in titin or myocardial stiffness (24). The pathophysiology of titin has been extensively reviewed elsewhere (36-38). We address titin-based therapeutic approaches in "cGMP—signaling" section.

Active cardiomyocyte relaxation

Whereas passive mechanical properties of cardiomyocytes are evaluated in skinned myofibers obtained from animals and from human myocardial biopsies ("the role of titin in impaired cardiomyocyte relaxation" section), examination of active contraction and relaxation during ECC requires access to intact and functional myocardium and isolated cardiomyocytes (quantitative measurements of Ca^{2+} and Na^+). Due to limited access to such samples from HFpEF patients, current data on ECC is mainly based on animal models. Advantages and limitations of currently available models have been comprehensively discussed recently (39).

Altered cytosolic Ca^{2+}- and Na^+-signaling

Cardiomyocyte contraction and relaxation is governed by the shape of the cytosolic CaT activating the myofilaments. Ca^{2+} release into the cytosol from the sarcoplasmic reticulum (SR) through ryanodine receptors (RyR2) is triggered by Ca^{2+} influx through L-type Ca^{2+} channels (LTCC) during the action potential (Ca^{2+}-induced Ca^{2+} release) and is highly controlled within intracellular microdomains (dyads). Cytosolic Ca^{2+} removal is achieved mainly by the ATP-dependent SR Ca^{2+} pump (SERCA) and to a lesser extent by extrusion via the sarcolemmal Na^+/Ca^{2+} exchanger (NCX) balancing the influx of Ca^{2+} via LTCC during the cardiac cycle. Cardiomyocytes from end-stage HFrEF are characterized by increased diastolic $[Ca^{2+}]_i$, reduced peak $[Ca^{2+}]_i$, a slower cytosolic Ca^{2+} removal and reuptake into the SR and a lower SR $[Ca^{2+}]$. Most consistently related is a reduced activity of SERCA and increased SR Ca^{2+} leak through RyR2. In recent years, with the development of animal models more closely mimicking the co-incidence of relevant triggering co-morbidities *in vivo*, distinct and more diverse cardiomyocyte phenotypes have been described in HFpEF. As summarized in *Table 1* alterations in cytosolic $[Ca^{2+}]$ have been observed in a variety of models in isolated cardiomyocytes *ex vivo*, indicating an adaptation of cardiomyocyte ECC in HFpEF even in the absence of the systemic metabolic and hemodynamic challenges present *in vivo*. Levels of diastolic $[Ca^{2+}]$ are often increased which not only reflects impaired relaxation but also influences the effects of Ca^{2+} release following depolarization (50). Cytosolic $[Ca^{2+}]$ decay is often prolonged, suggesting a contribution of prolonged active relaxation to diastolic dysfunction. In support, a close correlation between the $[Ca^{2+}]$ decay constant in isolated cardiomyocytes and the isovolumetric relaxation time *in vivo* has been documented in a cardiorenal HFpEF model (47). SR Ca^{2+} leak may be a sign of advanced stages of HFpEF (47). In contrast to end-stage HFrEF, the CaT amplitude {or SR $[Ca^{2+}]$, where determined} is not generally reduced and maybe even increased thus resembling the findings in "compensated" LV hypertrophy (51). Indeed, in a novel hypertrophic heart rat model without hypertension, diastolic dysfunction and congestion were associated

Table 1 Altered cardiomyocyte Ca^{2+} homeostasis in different preclinical models of HFpEF

Model	Hypertrophy without HTN	HTN	DM	CKD	Obese	LVEDP increased/ congestion	Rest			Stress			SR Ca	SR Ca leak	Reference
							Basal Ca	CaT amplitude	CaT decay rate	Basal Ca	CaT amplitude	CaT decay rate			
Rat: NXT		x		x		x	↔	↔	↓				↓	↑	(39,40)
Rat: ZSF1		x	x	x	x	x	↑	↔	↓						(41)
Rat: SHR		x				x	↑	↔	↓		↔	↔			(42)
Rat: AB		x				x	↑	↑	↓				↑	↑	(43)
Rat: AB		x					↔	↓	↔	↓	↓	↓	↓		(44)
Rat: ZDF		x	x		x	x	↔	↑	↔				↔		(45)
Rat: NXT + DOCA		x		x		x	↔	↔	↔						(46)
Rat: HHR	x					x	↑	↑	↓						(47)
Pig: AB		x				x	↔	↓	↓						(48)
Human: CAB		x				x		↔		↑					(49)

x, clinical condition; ↔, unchanged *vs.* control; ↑, increased *vs.* control; ↓, decreased *vs.* control. HFpEF, heart failure with preserved ejection fraction; HTN, hypertension; DM, diabetes mellitus; CKD, chronic kidney disease; LVEDP, left ventricular end-diastolic pressure; CaT, Ca^{2+} transient; SR, sarcoplasmic reticulum; NXT, subtotal nephrectomy; ZDF, Zucker diabetic fatty rat; SHR, spontaneously hypertensive rat; ZSF1, ZDF crossed with SHR; AB, aortic banding; HHR, hypertrophic heart rat; DOCA, chronic deoxycorticosterone acetate treatment; CAB, coronary artery bypass with HFpEF.

with increased LTCC influx and CaT amplitudes which may indicate a compensatory increase in inotropy in response to fibrotic hypertrophic remodeling (40). On the other hand, modeling of human myocardium suggests that a concentrically hypertrophied ventricular wall can maintain a preserved EF even despite reduced sarcomere shortening at the cardiomyocyte level (41) as implicated by reduced CaT amplitudes observed in some studies (*Table 1*).

It must be noted that only a minority of studies evaluated the functional response of cardiomyocytes from HFpEF models on physiological stressors (e.g., pacing frequency, adrenergic stimulation). Thus, more data is needed to elucidate the heterogeneity of the cardiomyocytes' functional response to exercise-related stimuli in HFpEF.

As opposed to end-stage HFrEF, a uniform pattern of expression of Ca^{2+} handling proteins has not emerged in HFpEF, but different modalities of post-translational regulation (e.g., oxidation, glycation, *S*-nitrosylation, phosphorylation) may contribute to altered Ca^{2+} trafficking, probably depending on HFpEF etiology (42,43). Also, increased cytosolic Ca^{2+} buffering slows Ca^{2+} trafficking and may decelerate Ca^{2+} decay and increase diastolic Ca^{2+} especially at higher heart rates (44,50).

Intracellular $[Ca^{2+}]$ is closely linked to $[Na^+]$ via the NCX which contributes to cytosolic Ca^{2+} removal even more in larger species and humans as compared to rodents (45). Increased $[Na^+]$ near the NCX can strongly influence the balance of transsarcolemmal Ca^{2+} fluxes and may thus contribute to altered cardiomyocyte relaxation in HFrEF (46,48). Potential mechanisms leading to intracellular Na^+ accumulation in cardiomyocytes include late Na^+ influx via fast (Nav1.5) Na^+ channels (INa,late), increased activity of the Na^+/H^+ exchanger (NHE1), reduced activity of the ATP-driven Na^+/K^+-pump (NKA), and influx via the sodium glucose co-transporter (SGLT1), the $Na^+/K^+/Cl^-$ co-transporter (NKCC1) or via less selective cation channels (e.g., TRP-channels). NCX undergoes complex mode shifts (forward/reverse) during the same cardiac cycle and may itself be a relevant source of Na^+ influx into cardiomyocytes (49). However, the role of $[Na^+]_i$ as a driver for diastolic dysfunction and the relevance of any of the mentioned mechanisms of Na^+ accumulation in cardiomyocytes in HFpEF is still under debate (47,52,53).

While experimental data, also in human myocardium, support the concept to treat diastolic dysfunction by inhibition of INa,late in a variety of cardiac disease models (54-57), the INa,late inhibitor ranolazine failed to improve diastolic function acutely and after 14d in a small cohort of patients with HFpEF in a clinical phase IIa proof-of-concept study (RALI-DHF) (58). In a more recent study, muscle strips from the LV anterior wall (epicardial biopsies) of patients with hypertensive heart disease and HFpEF showed a prolonged relaxation time and a rate-induced increase in diastolic $[Ca^{2+}]$. However, no frequency-dependent increase in cytosolic $[Na^+]$ or altered expression of Na^+-/Ca^{2+} handling proteins were detected, and neither the acute application of ranolazine, amiloride (inhibiting NHE1), or furosemide (targeting NKCC1) improved cardiomyocyte relaxation (59). These results have been interpreted to argue against a role of increased cardiomyocyte $[Na^+]_i$ in HFpEF (16,59). However, the complex regulation of sarcolemmal $[Na^+]$ fluxes may not be reflected in the bulk cytosol (60,61), and the contrasting findings in different models warrant a more quantitative assessment of intracellular $[Na^+]$ dynamics and the evaluation of long-term adaptation of the cardiomyocyte to $[Na^+]$ influx modulation in different etiologies of HFpEF.

Myofilament properties

HCM is often associated with global hypercontractility but also diastolic dysfunction as characterized e.g., by increased LV end-diastolic pressure or LA remodeling, which may result in overt HFpEF (20). Depending on the underlying gene mutation, different micro- and macroscopic myocardial HCM phenotypes have been characterized (21). While myocardial passive stiffness is increased (by interstitial fibrosis and increased wall thickness), these effects are likely sequelae of dysfunctional sarcomeres. At the myofilaments, specific mutations have been associated with myofilament disarray with impaired Ca^{2+} cycling, an increased energy cost of contraction and/or pathologically increased myosin-actin binding during relaxation and even in the resting state (21). Based on the latter, a novel therapeutic approach currently in clinical trials investigates the effects of a first-of-its class myosin-ATPase inhibitor, mavacamten, in patients with symptomatic HCM or hypertrophic obstructive cardiomyopathy (HOCM) (62-64). First results of recent clinical phase 2 studies suggest good tolerability and improvement in some parameters of diastolic dysfunction (62,64).

While myofilament properties have been extensively studied in hypertrophic and dilated cardiomyopathy (65), the contribution of altered Ca^{2+} sensitivity and cross-bridge cycling to contractile dysfunction in other, more common etiologies of HFpEF is less well understood. Binding of Ca^{2+} to the troponin complex of the thin filament depends on the dissociation constant of troponin C (TnC). Adrenergic, PKA-dependent phosphorylation of troponin I (TnI, at Ser23/24) and cardiac myosin-binding protein C (cMBP-C) can reduce myofilament Ca^{2+} sensitivity by an altered TnC-TnI interaction and thus enhance relaxation of the myofilaments (66,67). Myosin-ATPase forms the motor of cross-bridge cycling by converting energy into myofilament motion. In HFrEF, an activator of myosin-ATPase is currently evaluated in clinical trials to improve CO without changing the kinetics of contraction (68), whereas inhibition of a hypercontractile myosin-ATPase in genetic cardiomyopathies has been shown to prevent hypertrophic remodeling (69). The role of myosin-ATPase in HFpEF has not yet been explored.

In one of the few studies on active myofilament function in HFpEF, the expression of the sarcomeric proteins cMyBP-C, cTnI, and cardiac myosin light chain 2 (cMLC2) were found unaltered in old dogs with hypertension-induced LV hypertrophy and diastolic dysfunction (70). However, phosphorylation of cMyBP-C (–80% at S282), TnT, TnI, and MLC2 (–70% at S19) was reduced resulting in increased Ca^{2+} sensitivity, reduced maximal activated tension, and slowed relaxation. Interestingly, PKA activity was not different but expression of phosphatases (PP1 and PP2a) was increased. Reduced cMYBP-C phosphorylation has been directly linked to diastolic dysfunction (71) and its dysregulation may contribute to HFpEF in the aging heart (72).

Lovelock *et al*. also reported increased myofilament sensitization (DOCA HFpEF rat). In this study, glutathionylation rather than phosphorylation of MyBP-C was associated with cardiomyocyte dysfunction (73). Analogously a variety of different post-translational modifications at the level of the myofilament proteins have been associated with diastolic dysfunction, including among others glutathionylation, methylglyoxal modification or oxidation, highlighting the variability and complexity of upstream signaling pathways (74-76).

Metabolic dysregulation

Metabolic dysregulation—i.e., the clinical alterations of diabetes mellitus, hypercholesterinemia and adiposity—is
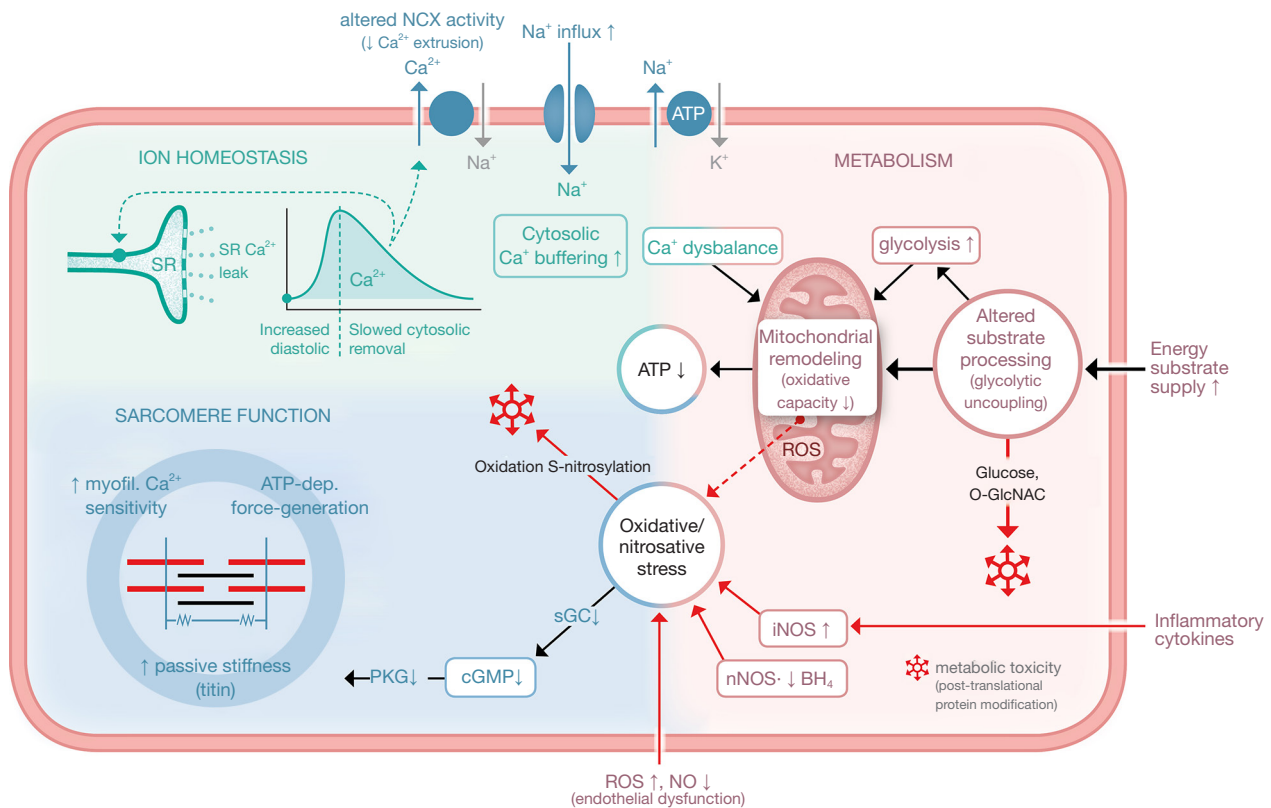
Figure 1 Mechanisms of cardiomyocyte dysfunction in HFpEF. Cardiomyocyte phenotypes include altered ion homeostasis (increased cytosolic Ca^{2+} and Na^{+} load), increased myofilament Ca^{2+} sensitivity, slower sarcomere relaxation and increased passive stiffness linked to reduced cGMP-PKG-mediated signaling. Mitochondrial oxidative capacity is reduced due to dysregulated intracellular metabolic processing despite an increased external supply with energy substrates. ROS (from mitochondria and external sources) and reduced NO availability contribute to increased oxidative and nitrosative stress and metabolic toxicity. HFpEF, heart failure with preserved ejection fraction; ROS, reactive oxygen species; NO, nitric oxide.

closely linked to HFpEF and within this population conveys an even worse prognosis (7,11,77). Adiposity, in particular, is an independent risk factor for the manifestation and progression of HFpEF (78,79). A considerable number of patients with HFpEF [24–45% (11)] suffer from type 2 diabetes mellitus promoting inflammation, increased interstitial fibrosis and collagen stiffening (39). At the cellular level, several aspects of metabolic dysregulation may contribute to HF (80), including an increased supply of the heart with energy substrates ["fuel overload" (81)], accumulation and dysfunctional processing of energy substrates within the myocardium (82,83) (*Figure 1*), and metabolic imbalance in peripheral tissue due to reduced CO and consequential reduced oxygen utilization in the peripheral tissue (84,85).

In healthy cardiomyocytes, the vast majority of ATP (>90%) is generated in the mitochondria by oxidative phosphorylation of fatty acids (FAs, 60%) or glucose (40%) (82). Increased glycolysis in combination with unchanged glucose oxidation (i.e., glycolytic uncoupling) was observed early in DOCA HFpEF rats (86). Collectively, the majority of current clinical and experimental studies in diabetic/obese as well as non-diabetics suggest that development of HFpEF (as opposed to HFrEF) is accompanied by increased FA oxidation and reduced glucose oxidation [see (83) for a comprehensive review]. Increased FA uptake is also observed in RV dysfunction [see Agarwal *et al.* in this issue (doi: 10.21037/cdt-20-404)]. Glucose oxidation is more effective, i.e., it yields more high-energy phosphate bonds per mol O_2 than FA, whereas FA yield

more energy per gram substrate than glucose if oxidation is not limiting (87). Comparison of ZSF-1 (diabetic obese) and DOCA + high salt diet (hypertensive) rat HFpEF models suggests differential regulation of oxidative phosphorylation depending on the HFpEF trigger (88). Also, in a porcine model of HFpEF induced by aortic banding, magnetic resonance spectroscopy imaging indicated a switch from FA towards glucose substrate utilization in the myocardium (89), again highlighting the complexity of HFpEF(-like) phenotypes. As a result of metabolic dysregulation, data from animal experiments suggest a decrease in cardiac (and peripheral) mitochondrial ATP production in hypertrophic hearts with diastolic dysfunction (90). In addition, structural changes associated with a reduced availability of functional mitochondria have been described in HFpEF (91).

In patients with HFpEF the ratio between cardiac creatine phosphate and adenosine triphosphate is reduced suggesting a reduced energy reserve (92).

In HFrEF mitochondrial remodeling has been linked to an increased mitochondrial production of reactive oxygen species (ROS). It is currently unclear whether mitochondrial ROS production in cardiomyocytes is increased in HFpEF. Another source for ROS is uncoupling of nitric oxide synthase (NOS) by a HFpEF-related reduction of the co-enzyme biopterin (93). Increased ROS add to oxidative/nitrosative stress and may interfere with a variety of intracellular signaling pathways (e.g., via CaMKII) by post-translational protein modification (*Figure 1*). ROS formation may be less evident in early HFpEF (94). In light of these findings, therapeutic approaches targeting mitochondria (i.e., to stimulate mitochondrial ATP production or reduce ROS) are currently evaluated (85). It has been proposed but not yet clinically validated that NO-cGMP related signaling may also increase the quantity of functionally active mitochondria thus potentially improving myocardial oxygen consumption (95). Interestingly, mitochondrial dysfunction has also been demonstrated in models of RV failure as reviewed by Agarwal *et al.* in this issue (doi: 10.21037/cdt-20-404).

Mitochondrial Ca signaling

In cardiomyocytes ATP consumption (mainly by the myofilaments) is tightly coupled to ATP production in the mitochondria via Ca^{2+} fluxes from the cytosol to the mitochondria (96). While disruption of the tight coupling between the cytosolic Ca^{2+} release units may contribute to impaired myofilament energy supply in HF, high mitochondrial Ca^{2+} exposure (as in myocardial ischemia with cytosolic Ca^{2+} overload) induces cardiomyocyte

apoptosis (97). In pressure overload-induced LV remodeling this delicate balance may be decisive for the transition from compensated hypertrophy to HF (98). Mitochondrial signaling in HFpEF is not yet well understood. In a porcine aortic banding model, mitochondria from HFpEF hearts were more susceptible to Ca^{2+} overload (99). In addition, a recent study showed that increased cytosolic Ca^{2+} turnover in HFpEF (see "altered cytosolic Ca^{2+}- and Na^+-signaling" section) was associated with increased mitochondrial Ca^{2+} load leading to reduced oxidative capacity and mitochondrial swelling. Based on these early data, protecting mitochondria from the cytosolic Ca^{2+} challenge should be evaluated as a therapeutic approach in HFpEF.

HFpEF—alterations in signaling

Systemic inflammation

Inflammation has been epidemiologically and mechanistically linked to HFpEF (100). Broadly identified as a non-specific defensive response of the organism to threats, inflammatory pathways are active in HFpEF and, importantly, contribute to its pathogenesis.

HFpEF is a syndrome driven by multiple comorbidities and conditions (27). Virtually, all of these (e.g., obesity, hypertension, aging etc.) are characterized by a status of subtle, chronic systemic inflammation contributing to the overall complex inflammatory activation in HFpEF. Although both the clinical significance and specific pathways of inflammatory alterations in HFpEF are yet to be fully determined, accumulating clinical and experimental evidence suggest that immune dysregulation is critical in the pathophysiology of HFpEF.

Clinical evidence of HFpEF as inflammatory disease

Some of the most striking evidence supporting a role for inflammation as key element of HFpEF progression are based on circulating biomarker discovery. Classical biomarkers of myocyte stretch and necrosis (NP and troponins) have less diagnostic value in HFpEF, compared to HFrEF (101). On the other hand, increased circulating levels of canonical inflammatory cytokines [e.g., tumor necrosis factor-alpha, soluble tumor necrosis factor-receptor I (sTNFRI), interleukin (IL)-6 and IL-1] were found to be predictive of incident HFpEF but not HFrEF (102-104). More recently, a European network analysis of circulating biomarkers in HF subjects revealed a unique biomarker preponderance related to inflammatory response, leukocyte

migration and cell adhesion in HFpEF patients (105).

Multiple phenotypes of HFpEF can be identified and categorized in different phenogroups that only partially overlap in terms of prevalence of comorbidities, pathophysiological mechanisms and clinical outcomes. The use of machine learning techniques has recently allowed the identification of distinctive biomarker profiles in different HFpEF phenogroups (102,106). Of interest, the presence of metabolic alterations seems to identify the phenogroup(s) with the greatest inflammatory activation. Specifically, increased adiposity has been associated with clusters of biomarkers related to immune activation and inflammation in specific HFpEF subgroups. The increase in circulating biomarkers of inflammation in HFpEF is supportive of the multiorgan, systemic inflammatory involvement in this syndrome. In addition, inflammatory activation (i.e., infiltrating immune cells and increased levels of critical mediators of inflammation) was demonstrated in HFpEF endomyocardial biopsies (30,107).

Based on these findings, inhibition of inflammation has been explored as therapeutic strategy in HFpEF. For example, blockade of IL-1 signaling in HFpEF with anakinra has been tested in the D-HART and D-HART2 clinical studies. While in the pilot D-HART study, HFpEF patients treated with anakinra showed improved aerobic exercise capacity (108), these findings were not confirmed in the subsequent phase II study (109). Failure of this anti-cytokine therapeutic approach might be attributable to the heterogeneity of unselected HFpEF population. The accurate phenotypic sub-classification of HFpEF patients together with the characterization of specific inflammatory pathways active in different HFpEF subgroups will be critical to identify clusters of HFpEF patients that may benefit most from anti-inflammatory therapeutic approaches.

Inflammatory-driven pathogenic pathways in HFpEF

The clinical evidence for HFpEF as an inflammatory disease is corroborated by preclinical studies focusing on the mechanistic aspects of this syndrome. Notably, the current knowledge is limited to inflammatory mediators and aspects of innate immunity, whereas the involvement and extent of alterations of adaptive immunity in HFpEF is still largely unknown (110).

In HFpEF, inflammation has been implicated in promoting coronary microvascular endothelial dysfunction and in the perturbation of NO availability, two hallmarks of myocardial alterations in HFpEF. Increased expression of adhesion molecules in the inflamed coronary endothelium drives the recruitment and infiltration of macrophages and other inflammatory cells promoting the development of interstitial fibrosis, which together with cardiomyocyte stiffening, contributes to diastolic dysfunction in HFpEF (111). For example, hypertension-induced diastolic dysfunction in mice causes the expansion of cardiac macrophage pools associated with the increased production of pro-fibrotic IL-10 which, in turn, amplifies and perpetuates diastolic dysfunction (112). Yet, the precise molecular events triggering the contribution of immune cells to diastolic dysfunction in HFpEF are still unknown.

Comorbidities-driven inflammation in HFpEF severely impacts on NO bioavailability in this syndrome. Inflammation increases oxidative stress and the production of ROS in HFpEF hearts, converting, in part, NO into peroxynitrite and other NO-related reactive species. In line with this notion, the presence of nitrosative stress in HFpEF has been identified as a major driver of this syndrome (113) (*Figure 1*).

The inflammatory-dependent reduction of NO bioavailability lowers the intracellular levels of cGMP, resulting in cardiomyocyte stiffening and impaired cellular relaxation (see "the role of titin in impaired cardiomyocyte relaxation" and "cGMP—signaling" sections).

Dysregulated NO production in HFpEF also involves the upregulation of inducible NOS (iNOS), a major inflammatory mediator, as shown in the myocardium of clinically relevant model(s) of HFpEF as well as in HFpEF human endomyocardial biopsies (72,107). The resulting nitrosative stress impacts on cardiomyocyte function at many levels perturbing fundamental signaling pathways involved in cardiomyocyte responses to chronic stress (*Figure 1*). Interestingly, the role of iNOS in promoting cardiomyocyte dysfunction has been associated with metabolic syndrome and hypertension, comorbidities present in the majority of HFpEF patients. This suggests iNOS as a crucial effector of metabolic stress-induced inflammation—i.e., metabolic inflammation or metainflammation—and thus, as a common mechanism between HFpEF and other metabolic diseases that might present a valuable therapeutic target across many inflammatory-driven disorders.

Neurohumoral activation

A recent clinical study suggests that neurohumoral activation—as quantified by elevated plasma renin activity, aldosterone or catecholamine levels—is less prevalent
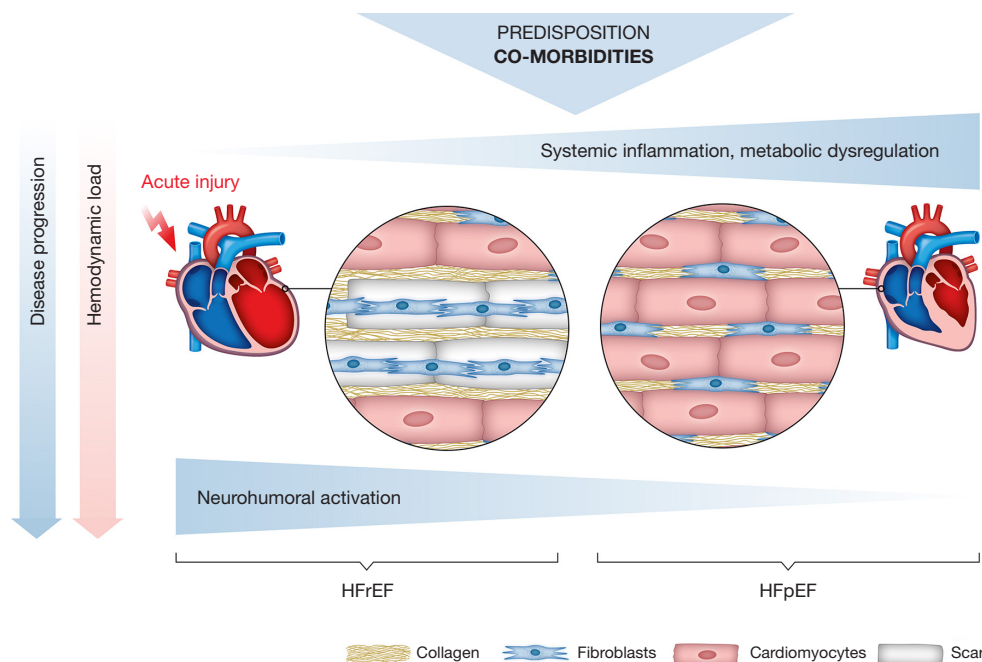
Figure 2 Myocardial remodeling in HFrEF and HFpEF. HFrEF, heart failure with reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction.

in HFpEF *vs.* HFrEF (114). Similarly, HFpEF was not associated with significant RAS activation as measured by ACE activity (94). These observations agree with earlier results indicating that neurohumoral activation (RAS and sympathetic) in HF is related to the degree of reduction in LV EF (115,116). Along similar lines, plasma aldosterone levels correlate with LV hypertrophy but not (independently) with LV diastolic dysfunction in patients with arterial hypertension (117). These findings may in part explain why RAS antagonists have failed to achieve survival benefit in HFpEF clinical trials (4,118). Interestingly, despite effectively reducing arterial blood pressure, RAS-inhibition is also not associated with improved LV remodeling in patients with preserved EF (119). Taken together, current evidence suggests that RAS activation may be present in some HFpEF patients but is likely not a relevant driver for diastolic dysfunction or increased morbidity and mortality (*Figure 2*). The role of aldosterone antagonists in HFpEF, however, remains to be determined (120).

In line with above findings on neurohumoral activation, betablockers have not been found beneficial in clinical studies in HFpEF patients, and maybe even harmful by worsening impaired chronotropic responses in some patients (121). In fact, withdrawal of pre-existing betablocker therapy in HFpEF patients is currently investigated as a therapeutic approach (122).

cGMP—signaling

In HFpEF, increased myocardial passive stiffness, in large parts mediated by the sarcomeric protein titin, has been associated with reduced activity of the cGMP-protein kinase G-signaling cascade in cardiomyocytes. Physiologically NO (from intra- and extracellular sources, *Figure 1*) and NP stimulate guanylyl cyclases (GCs) to maintain cGMP homeostasis. Increased levels of NP are considered a protective response in HF (123). In HFpEF, however, reduced bioavailability of NO, reduced efficacy of NP, and direct impairment of GC function (by oxidative stress) synergize to cause an intracellular lack of cGMP. Based on encouraging experimental data, several pharmacological approaches to increase cGMP were evaluated in clinical trials: soluble GC stimulators/activators, NO donors, inhibitors of the degradation of either cGMP (PDE5-inhibitors) or BNP (sacubitril/valsartan). To date, none of these clinical approaches has resulted in prognostic benefits in HFpEF patients, but stimulation of the cGMP-PKG-dependent pathway remains an attractive strategy as discussed in (124).

Right ventricular dysfunction (RVD) in HFpEF

Clinical prevalence and prognostic value

RVD, often in conjunction with pulmonary hypertension (PH), is commonly observed in patients suffering from HFpEF and closely related to worse clinical outcomes evident as increased hospitalization rates and a considerable increase in overall mortality as compared to HFpEF patients without RVD (125-127) (for an in depth review on RVD mortality see also Sanders *et al.* in this issue, doi: 10.21037/cdt-20-450). Therefore, detailed and longitudinal RV phenotyping of HFpEF patients is warranted to identify individuals at higher risk for adverse cardiac events or on a declining trajectory.

In the past, only a few studies have systematically screened for RVD in patients with HFpEF with considerable variation in reported prevalence between and even within studies. Puwanant and colleagues detected impaired RV function, defined as a decline in echocardiographically-assessed RV fractional area change (FAC) below 45%, in approximately 33% of a total of 51 HFpEF patients (128). Yet, when the authors defined RVD as tricuspid annular plane systolic excursion (TAPSE) of <15 mm or peak systolic tricuspid annular tissue velocity S' <115 mm/s, which both assess RV systolic performance, 40% and 50% of HFpEF patients, respectively, were classified with RVD (128). A larger study of 309 HFpEF patients, however, reported RVD in only 20% of HFpEF patients, albeit using a slightly higher TAPSE of <15.9 mm as cut-off (129). In yet another cohort of 201 HFpEF patients, speckle-tracking echocardiography-derived RV strain identified RV systolic and diastolic abnormalities in 75% and 48% of patients, respectively (130). Finally, in a community-based study from Olmsted County, Minnesota, of 500 patients identified with HFpEF 35% and 21% showed signs of RVD as defined by a TAPSE of <16.0 mm or by semi-quantitative two-dimensional echocardiography (i.e., visual assessment of RV contractility, and comparison of RV to LV size, summarized on a four-point ordinal scale) (131).

The considerable variability between studies in the reported prevalence can be in part attributed to differences in study populations (age, sex, comorbidities, medications), deviating study protocols for clinical assessment of RVD, and use of different cut-off values (132). While these variabilities preclude a precise estimate of the prevalence of RVD in HFpEF patients, one can conclude that RVD is a common comorbidity in HFpEF, with an average prevalence of 30% based on TAPSE criteria. As such, RVD should deserve special attention in HF phenotyping (133).

Importantly, in all of the above studies RVD was diagnosed based on echocardiographic assessment of RV morphology and function, rather than invasive measurements of systolic, mean, and diastolic pulmonary artery pressure (PAP) as well as pulmonary capillary wedge pressure (PCWP) as a direct reflection of increased RV afterload, to which the RV is particularly vulnerable. Although the etiology of increased RV afterload in HFpEF initially is primarily attributable to increased left-atrial (LA) filling pressures and subsequent retrograde congestion of the pulmonary veins, capillaries and arteries, the resulting PH is frequently complicated by secondary pulmonary vascular remodeling (134) (for discussion of the underlying mechanisms please see below). As such, assessment of the transpulmonary pressure gradient (TPG; mean PAP-PCWP) or diastolic pressure gradient (DPG; diastolic PAP-PCWP) constitutes an important diagnostic test for left and right heart disease (135). Accordingly, state-of-the-art diagnostic HFpEF guidelines recommend the assessment of PCWP for a subgroup of patients as part of a diastolic stress testing (136). Given the risks associated with right heart catheterization, invasive hemodynamic phenotyping of the right heart is, however, scarcely performed. Indeed, simultaneous left and right heart hemodynamics may be warranted in some patients as outlined by Hansmann *et al.* in this issue (doi: 10.21037/cdt-20-483).

The prognostic relevance of RVD in HFpEF patients was highlighted by the group of Margaret Redfield in the already mentioned Olmsted County study (131) demonstrating that all-cause and cardiovascular mortality, as well as time to first or multiple HF hospitalizations are associated with increased systolic PAP, decreased TAPSE, and semi-quantitative confirmation of RVD. These findings were corroborated in a subsequent study which reported an almost 10-fold higher 1-year all-cause mortality for HFpEF patients with RVD compared to those without (41.7% *vs.* 4.8%) (127). On the basis of these data, it seems compelling that HF phenotyping guidelines need to implement standardized RV phenotyping protocols to identify patient populations who evidently face the greatest risk for critical events.

Potential mechanisms of RV dysfunction

HFpEF causes "passive" backward congestion of blood pressure from the left ventricle into the pulmonary circulation and ultimately, the RV; however, with

HFrEF	HFpEF
Lv eccentric hypertrophy	LV concentric hypertrophy
↑ LV cavity size	↓ LV cavity size
↑ EDV	↓ EDV
Transiently maintained SV	↓ SV
Balanced ventricular output	Ventricular output imbalance
PH-LHD (68%)	PH-LHD (83%)
Medium probability for RVD	High probability for RVD

Figure 3 RVD in HFpEF *vs*. HFrEF. HFpEF is characterized by concentric LV hypertrophy. In turn, LV cavity size, EDV, and ultimately SV decrease. The resulting ventricular output imbalance between LV and RV increases the prevalence of PH-LHD and hence, the probability of RVD. Conversely, HFrEF causes LV dilatation, increasing LV cavity size and EDV. This enables the LV to transiently maintain a stable SV despite a reduced EF. As a consequence, ventricular output imbalance, PH-LHD and ultimately RVD are less common in HFrEF. RVD, right ventricular dysfunction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LV, left ventricular; EDV, end-diastolic volume; SV, stroke volume; PH-LHD, pulmonary hypertension associated with left heart disease.

advancing HFpEF severity and duration additional "active" mechanisms such as lung vascular remodeling and LV-RV interaction come progressively into play. While normal RV function is often maintained during mere passive congestion—frequently referred to as "isolated post-capillary PH" (IpcPH)—additional "active" pulmonary vascular remodeling will result in "combined post- and pre-capillary PH" (CpcPH) which directly determines the extent and course of RVD (137,138).

Postcapillary pressure overload
The "passive" component of backwards congestion in left heart disease (LHD) is based on the basic physical principle of pressure transmission along communicating pipes that ultimately results in PH. While PH was originally, yet somewhat arbitrarily, defined as mean PAP ≥25 mmHg, a series of seminal studies published in the past 5 years revealed that individuals with a mean PAP of 21 to 24 mmHg are also at increased risk for poor outcomes (139,140). At the 6th World Symposium for Pulmonary Hypertension in Nice in 2018, these findings led to the recommendation to define a mean PAP ≥20 mmHg as new cutoff for diagnosing

PH (141). Of the 5 different groups of PH, the most common one is PH-LHD which in addition to an increase in mean PAP ≥20 mmHg is defined by a concomitant PCWP >15 mmHg (142-144). Remarkably, epidemiological data suggest that PH-LHD (and thus, presumably, also RVD) is more frequent in HFpEF (83%) (145) as compared to HFrEF patients (68%) (146). This differential prevalence is in line with basic physiological principles (*Figure 3*): The hallmarks of HFpEF are concentric LV hypertrophy and diastolic dysfunction; in contrast, HFrEF is characterized by systolic dysfunction and LV dilatation. As a result, end-diastolic volumes (EDVs) are considerably smaller in HFpEF as compared to HFrEF, and even though LV EF will by definition be higher, the resulting LV SV will be smaller in HFpEF as compared to HFrEF (147). Importantly, it is the difference in SV, not in EF that characterizes ventricular output imbalance. Given the more pronounced reduction in LV SV, it may thus come as little surprise that PH-LHD is apparently more frequent in HFpEF as compared to HFrEF.

The current nomenclature classifies "passive" postcapillary pressure overload as IpcPH, defined as PH-

Table 2 Hemodynamic definitions of PH

PH type	Hemodynamic definitions
PH-LHD	mPAP ≥20 mmHg; PCWP >15 mmHg
IpcPH	PVR ≤3WU; DPG <7 mmHg; C_{PA} ≥2.3 mL/mmHg
CpcPH	PVR >3WU; DPG ≥7 mmHg; C_{PA} <2.3 mL/mmHg

Modified from (138). PH-LHD, pulmonary hypertension associated with left heart disease; mPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; IpcPH, isolated post-capillary pulmonary hypertension; CpcPH, combined post- and precapillary pulmonary hypertension; PVR, pulmonary vascular resistance; WU, Wood units; DPG, diastolic pressure gradient; CPA, pulmonary artery compliance.

LHD with a pulmonary vascular resistance (PVR) ≤3 Wood units, a DPG <7 mmHg, or a pulmonary artery compliance ≥2.3 mL/mmHg (*Table 2*). Pathologically, IpcPH is primarily characterized by the typical signs of congestive HF, i.e., interstitial and subsequent alveolar lung oedema, which in case of capillary stress failure can advance to alveolar hemorrhage (148).

Precapillary PH

When "active" pulmonary vascular remodelling adds a precapillary component to the post-capillary pressure overload, IpcPH transitions to CpcPH defined as PH-LHD with a PVR >3 Wood units, a DPG ≥7 mmHg, or a pulmonary artery compliance <2.3 mL/mmHg (*Table 1*). The underlying changes in the pulmonary vasculature comprise both medial and intimal thickening across the entirety of small vessels in the lung (134,149,150). The combined "passive" and "active" effects of HFpEF and pulmonary vascular remodeling ultimately affect the function of the RV (for a comprehensive overview on the non-invasive and invasive assessment of RV hemodynamics see Bernardo *et al.* and Truog *et al.* in this issue, doi: 10.21037/cdt-20-479, doi: 10.21037/cdt-20-272). In response to the elevated afterload, the RV undergoes compensatory concentric hypertrophy and at its peak, is able to increase contractility by a factor of five (144). This phenomenon is commonly described as RV-PA coupling, which describes the energy transfer between RV contractility and arterial afterload, where RV contractility can be characterized by end-systolic elastance (Ees). Arterial afterload can be thought of as net arterial stiffness (Ea) (151,152). With disease progression, intraventricular pressure rises exerting increased stress on the RV wall inevitably leading to RV dilation. Increasing oxygen demand (required to pump against an increased

afterload) can no longer be matched by a decreasing oxygen supply as diastolic coronary perfusion is progressively reduced as a result of increased ventricular wall tension. In combination, these events trigger a downward spiral of ischemia, deteriorating contractility, failing RV CO and ultimately general decompensation (142).

LV-RV interaction

An additional characteristic feature of RVD in HFpEF patients is tricuspid valve (TV) regurgitation due to enlargement of the TV annulus - preferably at the level of the free wall as a consequence of RV dilation (153). Similarly or in parallel, regurgitation may be caused by papillary muscle displacement as a result of RV remodeling (154). Finally, TV regurgitation may be directly driven by pathological changes in cardiac geometry: The transmission of LV structural and locomotorical changes via the interventricular septum to the RV increases the risk of TV leaflet prolapse and affects the position of the septal leaflet up to a point where efficient valve coaptation is no longer possible (155,156). The resulting TV regurgitation will further contribute to RV dilation and impaired RV CO, thus adding to the vicious cycle of accelerating RV failure. The latter scenario also underlines that in addition to "passive" congestion and "active" pulmonary vascular remodeling, direct interaction between the RV and LV may drive RVD in HFpEF. Conversely, the progressive increase in RV afterload in CpcPH may further negatively impact on LV diastolic function, as indicated by the clinical finding that release of RV afterload by pulmonary thrombarterectomy markedly improves LV EDV and SV (157). This RV-LV interaction is probably mediated in large part through septal interdependence, as leftward displacement of the interventricular septum further impedes LV filling (158). Notably, RV-LV interdependence extends beyond the level of hemodynamic interactions (158), but may comprise local remodeling and pro-fibrotic effects at the ventricular hinge-points (159) as well as vascular information transfer via gap junction-mediated conducted responses that can propagate to the contralateral ventricle along the continuity of the shared RV-LV microvasculature.

Future molecular approaches to HFpEF

As outlined in this review, current clinical and experimental evidence supports the concept of inflammation contributing to HFpEF pathogenesis. However, a better understanding

of targetable signaling pathways is needed to translate these discoveries into novel therapeutic approaches. To this end, the careful phenotyping of clinically relevant animal models *in vivo* and *in vitro*, both at rest and during exercise/stress is mandatory in order to characterize a functional response rather than a steady state. While chronic inflammation sets the stage for cardiac remodeling, different co-morbidities likely trigger specific patterns of cellular adaptations which need to be delineated preferably in a structured comparison of animal models. Targeting well established pathways (e.g., cGMP-PKG) calls for novel approaches [such as inhibition of different PDEs (160) or sGLT inhibitors (161)]. Efforts to exploit identified mitochondrial-based therapeutic approaches (anti-ROS, pro ATP) are already underway (85). Cardiomyocyte ECC is tightly controlled and interventions targeting intracellular Na^+ and Ca^{2+} need to take chronic adaptation of ion homeostasis into account (52). Myocardial fibrosis—while prevalent in HFpEF—requires validation as therapeutic target using established (spironolactone) and novel substances with anti-fibrotic activity (162). Promoting a targeted therapeutic approach (6), a more in-depth clinical phenotyping including comprehensive imaging analysis of both ventricles (strain, LA and RV involvement) and multi-biomarker panels (163,164) will be the basis for HFpEF treatment with the goal to identify phenogroups of patients who may have the highest likelihood to benefit from a specific targeted therapy.

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Footnote

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