Chapter 8

From Comorbidities to Heart Failure with Preserved Ejection Fraction: A Story of Oxidative Stress

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Heart Failure with Preserved Ejection Fraction:
A Story of Oxidative Stress

by


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Running Title: Microvascular Inflammation in HFpEF
INTRODUCTION

Heart failure (HF) with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF) currently account for roughly equal proportions of HF[1]. The incidence of HFpEF increased rapidly during the past decades and is becoming the dominant form of HF[2]. Recently this was reappraised and it was shown that the incidence of HF decreased, but this was more pronounced for HFrEF than in HFpEF[1,3,4]. Also, since HFrEF patients benefit from therapeutic progress, many of these patients shifted to HFpEF for which there is no specific treatment[1]. Although various HFpEF trials conducted to date could be criticised for methodological shortcomings[5], a more serious weakness is our incomplete understanding of the pathophysiology of HFpEF[6].

HFpEF is characterised by a high incidence of non-cardiac comorbidities such as obesity, diabetes mellitus (DM), chronic obstructive pulmonary disease (COPD) and arterial hypertension, all of which are of prognostic importance and lead to increased morbidity and mortality in this elderly population[1]. This marked association suggests that comorbidities play a key role in HFpEF pathophysiology.

HFpEF is characterized by concentric left ventricular (LV) remodelling and diastolic dysfunction[7]. In addition, impairments in chronotropic reserve, atrial function, systemic and pulmonary vasculature, vasodilatation and many other factors are known to be involved[8]. Identifying and understanding these underlying mechanisms is essential to develop treatment strategies for HFpEF. In this review, we will provide an overview of HFpEF from diagnosis to pathophysiology, and we will highlight the importance of comorbidities in endothelial inflammation and oxidative stress as underlying pathophysiological mechanisms. Finally, we will touch upon some of the many treatment strategies that have been and will be studied in HFpEF.

DIAGNOSIS OF HFPEF

Diagnosing HFpEF remains challenging and requires signs or symptoms of congestion, preserved or mildly abnormal LV systolic function (EF>50%, end-diastolic volume index <97 ml/m²) and diastolic LV dysfunction (Figure 1)[9]. Diastolic LV dysfunction is defined as the inability of the ventricle to fill to a normal
How to diagnose HFpEF

Symptoms or signs of heart failure

Normal or mildly reduced left ventricular systolic function
LVEF > 50% and LVEDVI < 97 mL/m²

Evidence of abnormal LV relaxation, filling, diastolic diastolic stiffness and diastolic stiffness

Invasive Haemodynamic measurements
mPCW > 12 mmHg
or LVEDP > 16 mmHg
or \( \tau > 48 \text{ ms} \)
or \( b > 0.27 \)

TD
\( E/E' > 15 \)
15 > \( E/E' > 8 \)

Biomarkers
NT-proBNP > 220 pg/mL
or BNP > 200 pg/mL

Echo – bloodflow Doppler
\( E/A < 0.5 \) and DT \( > 280 \text{ ms} \)
or Ard-Ad > 30 ms
or LAVI > 40 mL/m²
or LVMi > 122 g/m² (m²)
or >149 g/m² (c²)
or Atrial fibrillation

Future improvements of this diagnostic algorithm should focus on comorbidity profile, exercise hemodynamics and new biomarkers. Adapted with permission from Paulus et al[9].

Figure 1. Diagnostic flowchart on ‘How to diagnose HFpEF’ in a patient suspected of HFpEF. LVEDVI, left ventricular end-diastolic volume index; mPCW, mean pulmonary capillary wedge pressure; LVEDP, left ventricular end-diastolic pressure; \( \tau \), time constant of left ventricular relaxation; \( b \), constant of left ventricular chamber stiffness; TD, tissue Doppler; \( E \), early mitral valve flow velocity; \( E' \), early TD lengthening velocity; NT-proBNP, N-terminal-pro brain natriuretic peptide; BNP, brain natriuretic peptide; \( E/A \), ratio of early \( (E) \) to late \( (A) \) mitral valve flow velocity; DT, deceleration time; LVMi, left ventricular mass index; LAVI, left atrial volume index; Ard, duration of reverse pulmonary vein atrial systole flow; Ad, duration of mitral valve atrial wave flow. Future improvements of this diagnostic algorithm should focus on comorbidity profile, exercise hemodynamics and new biomarkers. Adapted with permission from Paulus et al[9].

preload volume at low pressures. It can be diagnosed invasively by measurement of an increased pulmonary capillary wedge pressure (PCWP), LV end-diastolic pressure or prolonged LV isovolumic relaxation[9]. Doppler echocardiography guides non-invasive diagnosis with an \( E/E' > 15 \) (ratio of early transmitial diastolic flow velocity to tissue Doppler early mitral annular diastolic velocity). When \( E/E' \) is 8-15, other echocardiographic parameters (left atrial size, transmitial and pulmonary flow velocities, LV hypertrophy), atrial fibrillation and natriuretic
peptides (NP) may yield secondary evidence of diastolic dysfunction[9]. Future efforts to improve this diagnostic algorithm should focus on comorbidity profile, exercise hemodynamics (as discussed below) and new biomarkers.

However, many HFpEF patients only develop symptoms during (limited) exercise and are asymptomatic at rest - when echocardiography or cardiac catheterization are usually performed. It has been demonstrated that HFpEF patients are very sensitive to volume status and even a relatively small volume load with 0.6l of saline can make PCWP rise sharply[10]. Since many patients are on fluid and/or salt restriction before undergoing catheterization, measured values might underestimate actual pressures outside the clinical situation. Also, normal levels of natriuretic peptides do not rule out HFpEF[11]. Importantly, exercise during invasive measurements made filling pressures rise at even limited workloads, probably more accurately reflecting activities of daily living[12–14]. Moreover, exercise testing was whown to be more sensitive than saline loading to detect hemodynamic derangements indicative of HFpEF[15]. Furthermore, invasive exercise testing enables the clinician to detect mechanisms limiting exercise tolerance, such as prompt elevation of filling pressures, chronotropic incompetence[16], paradoxical rise of pulmonary vascular resistance[17] and inappropriate widening of arterio-venous oxygen content difference[18].

The cardiac and cardiovascular mechanisms that impair exercise tolerance in HFpEF, such as ventricular diastolic and systolic reserve function, heart rate reserve and rhythm, atrial dysfunction, stiffening of the ventricles and vasculature, impaired vasodilatation, pulmonary hypertension, endothelial dysfunction and peripheral abnormalities were recently extensively reviewed elsewhere[8].

**PATHOPHYSIOLOGICAL FINDINGS IN HFPEF**

HFpEF is characterized by concentric LV remodeling, diastolic dysfunction and increased myocardial stiffness[7,19]. These findings are associated with structural and functional changes in myocardial samples from patients and animal models. The most important findings are discussed below.

**Structural changes in HFpEF myocardium: the extracellular matrix**

The changes in the extracellular matrix (ECM) known to occur in HFpEF patients are largely determined by collagen and consist of an increased collagen volume
fraction (CVF), a relative abundance of the stiff collagen type 1 and more collagen cross-linking[20,21]. Inflammatory markers (such as interleukins 6 and 8), endothelial adhesion molecules [intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1], and markers of ECM turnover [matrix metalloproteinase 9 (MMP9), tissue inhibitor of metalloproteinase 1 (TIMP1)] are all increased in HFP EF[22]. As recently hypothesized, microvascular inflammation is probably the driving mechanism leading to proliferation of fibroblasts and myofibroblasts as a consequence of decreased NO bioavailability, which induces profibrotic effects and coronary microvascular rarefaction[6,23].

Besides changes in the ECM, structural differences between HFP EF and HFrEF cardiomyocytes were observed[24]. Cardiomyocyte diameters were larger in HFP EF than in HFrEF in absolute values and at corresponding levels of CVF[24]. This and other recent evidence from HFP EF biopsy samples suggest that, on top of the observed structural changes, cardiomyocytes functionally contribute to HFP EF pathophysiology[23,25]. Additionally, no changes in ECM structure were observed in a novel rat model, in which a HFP EF phenotype with diastolic dysfunction, pulmonary congestion and increased myocardial stiffness was induced by metabolic risk factors such as obesity and DM on top of arterial hypertension[26]. These findings indicate that functional changes in cardiomyocytes are important in HFP EF.

**Functional changes in HFP EF cardiomyocytes**

As already mentioned, cardiomyocyte diameters are typically larger typically in HFP EF than in HFrEF[24]. Also, cardiomyocyte stiffness was higher in HFP EF than in HFrEF and highest in HFP EF patients with DM[24,27]. Upon administration of protein kinase A (PKA), cardiomyocyte stiffness declined more in HFP EF than in HFrEF cardiomyocytes, suggesting that a phosphorylation deficit contributes to myocardial and diastolic stiffness in HFP EF.

The giant protein titin is the main determinant of myocardial stiffness in cardiomyocytes supporting diastolic distensibility. Titin modulates cardiomyocyte stiffness, via either phosphorylation or oxidation[28]. Titin can, for example, be phosphorylated by protein kinase A (PKA), upon -adrenergic stimulation, or by protein kinase G (PKG), upon activation by nitric oxide (NO) or natriuretic peptides (NP). PKA andPKG phosphorylation acutely increase titin compliance and potentially contributes to enhanced cardiac filling[28]. The reduced myocardial
stiffness upon titin phosphorylation by PKA and PKG suggests that the chronic phosphorylation deficit observed in HFP EF is most likely due to impaired PKA and PKG signalling. Indeed, both PKA and/or PKG could correct the high cardiomyocyte stiffness of HFP EF patients biopsy samples and animal models of HFP EF[24,26,27,29,30]. However, a HFP EF dog model showed no change in PKA activity, suggesting that PKG may be more important[31]. Indeed, PKG activity and cGMP concentration were reduced in LV myocardium of HFP EF patients and this was associated with increased myocardial nitrotyrosine levels[29]. Elevated nitrotyrosine is a marker of nitrosative stress as will be discussed below. Together, these findings suggest that downregulated cGMP-PKG-signalling is an important finding in HFP EF and cGMP-enhancing therapy could be useful.

Next to the cGMP-PKG-signalling pathway, recent studies suggest a role for proteotoxicity in cardiac dysfunction, as it was previously demonstrated in neurological diseases such as Alzheimer’s and Huntington’s disease[32]. The basis for this proteotoxicity resides in the failing quality-control and/or repair mechanisms to correct for aggregated or damaged proteins as a consequence of inflammation and oxidative stress, but it is also part of normal (cardiac) aging[32,33] and it can affect mitochondrial dysfunction. Knowledge about the role for proteotoxicity and mitochondrial dysfunction in relation to aging, inflammation and oxidative stress in HFP EF is still premature, but might explain the absence of benefit from different treatment strategies so far[34].

**COMORBIDITIES IN HFP EF PATIENTS**

**Prevalence**

Several comorbidities are highly prevalent in HFP EF patient populations. Coronary artery disease (CAD) has been reported in HFP EF, is associated with increased mortality and its prevalence ranges from 25% to 68%, depending on the methodology applied in two recent studies[35,36]. Unfortunately, non-invasive detection is still problematic with many false-positive and false-negative exercise tests[35]. Also, the proportion of patients reporting angina did not differ between patients with or without significant CAD[35], suggesting the presence of microvasculatory dysfunction with subsequent angina. Prospective studies are needed to define how to diagnose CAD in HFP EF patients in the absence of an
acute coronary syndrome, but for now, coronary angiography should probably be considered in case of recurrent or worsening HF[36].

Although waist circumference and overweight patients are often not reported in studies, one third of patients has a body mass index ≥30kg/m² [37,38]. DM prevalence ranges from 37 to 45% in various registries, while arterial hypertension shows an even higher prevalence, ranging from 76 to 96%[37–40]. Furthermore, around 1/3 of HFpEF patients suffer from COPD and 26-52% of patients have chronic kidney disease[38,40]. Moreover, many patients have more than one comorbidity and the number of comorbidities correlates with the prognosis[3,4]. Non-cardiac comorbidities induce a chronic, low-grade inflammatory state and oxidative stress, as will be discussed next.

Inflammation
The association between chronic, low-grade inflammation and the development of HF has been a topic of extensive research. Diverse inflammatory markers are associated not only with the development and diagnosis of HFpEF, but also with the prognosis of HFpEF patients[22,41]. Obesity with increased visceral adipose tissue induces several pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), monocyte chemoattractant protein 1 (MCP-1) and other chemokine ligands, all of which lead to monocyte recruitment and macrophage activation[42]. Increased peripheral inflammation, monocytosis, and monocyte differentiation to anti-inflammatory/pro-fibrotic M2 macrophages was shown to be associated with HFpEF in a population with a very high prevalence of metabolic comorbidities[43]. In addition, perivascular adipose tissue plays a major role in mediating vascular tone and (endothelial) inflammation through the interaction of perivascular adipocytes, immune cells, vascular endothelium and smooth muscle cells[44], effects also mediated by reduced expression of endothelial NO synthase (eNOS) and thus decreased NO synthesis, leading to reduced vasorelaxation[42]. In addition, obesity-associated inflammation also induces insulin resistance (IR)[42], an early step in the development of DM. In HFpEF patients, inflammatory changes could also occur in skeletal muscle and account for the impaired diffusion of oxygen to skeletal muscle during exercise[45] and the blunted systemic vasodilator response[46].
Oxidative stress

Inflammatory cytokines induce the production of the reactive oxygen species (ROS) superoxide (O$_2^-$) by NADPH oxidases (NOX), enzymes that are widespread in the human body (e.g. in macrophages, endothelial cells, vascular smooth muscle cells and cardiomyocytes)[47]. Increased myocardial activation of NOX was previously described in patients with dilated and ischemic cardiomyopathy[48,49]. Aortic banding in rats led to LV hypertrophy and HF and resulted in increased NOX expression in cardiomyocytes and endothelium[50].

At low concentrations, ROS play a physiological role in signaling cascades[51]. However, when ROS production increases or availability of antioxidants is insufficient, oxidative stress results with detrimental effects. Increased concentrations of ROS induce nonspecific damage to cellular membranes, proteins, and DNA, and is especially harmful in mitochondria since mitochondrial DNA and enzymes are highly susceptible to oxidative damage[52]. In the microvasculature, ROS are known to ‘uncouple’ endothelial nitric oxide synthase (eNOS, also known as NOS3)[53]. eNOS is found in the cardiovascular system and normally produces NO in its dimer state. However, the uncoupled monomer generates superoxide rather than NO and exacerbates the oxidative stress. Moreover, the NO generated reacts rapidly with superoxide to generate peroxynitrite (ONOO$^-$), which is a toxic, reactive nitrogen species with the ability to nitrate tyrosine residues and form nitrotyrosine[51]. The resultant decreased NO bioavailability was recently proposed to be essential in HFpEF development and is further discussed next[6] (Figure 2).

DECREASED NO BIOAVAILABILITY DRIVES HFPEF DEVELOPMENT

NO is essential in cardiovascular physiology and its function as an endothelial-derived relaxant of smooth muscle was described almost three decades ago[54]. NO regulates the activity of the sarcoplasmic reticulum (SR) Ca$^{2+}$-ATPase (SERCA), which adjusts Ca$^{2+}$ re-uptake into the SR, and hence inotropy[55]. NO also regulates the ryanodine receptors, responsible for the cytosolic Ca$^{2+}$-influx from the SR, and decreased NO bioavailability can change contractility[56].

Besides effects on contractile properties, NO can induce earlier LV relaxation and reduce end-diastolic stiffness[57]. This last mechanism functions via stimulation of cardiac soluble guanylate cyclase (sGC) receptors, which catalyze
the conversion of guanosine 5’-triphosphate (GTP) to cyclic guanosine 3’,5’-monophosphate (cGMP)[58]. NPs such as brain natriuretic peptide (BNP) increase cGMP via stimulation of particulate guanylate cyclase (pGC)[59].
cGMP is an ubiquitous intracellular second-messenger, vital to endothelial, vascular smooth muscle, and cardiomyocyte function[6,60]. It exerts its actions through cGMP-gated cation channels, cGMP-dependent protein kinases (PKG), and cGMP-regulated phosphodiesterases (PDEs), which in turn hydrolyze cGMP and other cyclic nucleotides[60].

In HFrEF, where NO bioavailability is low due to inflammation and oxidative stress, cGMP concentration and PKG activity decrease. Also, oxidative stress itself shifts sGC toward an oxidized, dysfunctional heme-free form that is unresponsive to both endogenous and exogenous NO[61]. Indeed, cGMP concentration and PKG activity were shown to be lower in myocardial samples from HFrEF patients compared to patients with HFrEF or AS and in vitro PKG normalized the increased stiffness of HFrEF cardiomyocytes[29]. As already mentioned, HFrEF animal models confirmed titin hypo-phosphorylation associated with stiff cardiomyocytes, which was normalized upon in vitro administration of PKG[26,30,31]. As a consequence, this disrupted NO-sGC-cGMP-PKG pathway in HFrEF might yield therapeutic targets in HFrEF[61], as discussed below (Figure 3).

THERAPEUTIC STRATEGIES

The past
The management of patients with HFrEF has improved considerably over the past decades and current guidelines from the joint European Society of Cardiology (ESC) and American College of Cardiology Foundation (ACCF) and the American Heart Association (AHA) on the management of HF provide the clinician with guidance[62,63]. In stark contrast, the guidelines sections related to HFrEF are sparse and are limited to symptomatic treatment of congestion with diuretics and the control of (metabolic) comorbidities such as arterial hypertension and DM. No treatment strategy studied to date in large HFrEF trials has proven to improve disease progression and survival, including betablockers[64], angiotensin-converting enzyme inhibitors[65], angiotensin receptor blockers[66,67], and digoxin[68]. An exhaustive enumeration of past trials in HFrEF was recently tabulated[69].
The present: PDE5 inhibitors and aldosterone antagonists in HFpEF

cGMP breakdown can be inhibited by the PDE5 inhibitor sildenafil with potential beneficial effects such as improved cardiac relaxation and LV reverse remodelling[70] (Figure 3). PDE5 inhibition is integrated in the guidelines for pharmacologic treatment of pulmonary arterial hypertension in adults, where it improves exercise capacity, functional status and hemodynamics [71,72]. When treated with sildenafil, pulmonary vascular resistance and right heart pressures decreased in HFrEF patients with secondary pulmonary hypertension (PHT) and long-term treatment improved functional status, exercise tolerance, LV diastolic function and cardiac geometry[73–75]. Many HFpEF patients develop PHT due to elevated left-sided filling pressures[76] and, vice versa, patients with unexplained PHT appear to have HFpEF[77]. In a small clinical study in HFpEF patients with PHT, treatment with sildenafil for 12 months improved LV diastolic function and reduced hypertrophy and pulmonary pressures[78]. However, these effects were not reproduced in a larger, long-term (24 weeks) trial of sildenafil (RELAX) in HFpEF patients. In this study, exercise capacity and clinical status did not improve and sildenafil failed to increase plasma cGMP concentrations or yield hemodynamic benefits[79]. The authors suggested these disappointing results were attributable to the relatively low right-sided heart pressures in their patient population compared with the earlier studies in HFrEF. In addition, plasma levels of N-terminal pro-BNP (NT-proBNP) and prevalence of atrial fibrillation were high, indicating that patients in the RELAX trial were at an advanced stage of HFpEF and therefore less likely to benefit from a limited strategy involving only inhibition of cGMP breakdown[61]. Since decreased myocardial cGMP concentration is an important finding in HFpEF, stimulation of cGMP production is an interesting therapeutic strategy in HFpEF as will be discussed in more detail below.

In patients with HFrEF, regardless of the cause, the aldosterone antagonists spironolactone and epleronone reduce total and cardiovascular mortality[80–82] and are recommended in current HF guidelines[62,63]. Aldosterone plays an important role in the pathophysiology of all forms of HF and is implicated in vascular dysfunction, endothelial inflammation, increased sodium retention and volume load, hypertrophy and fibrosis[83,84]. Specific to HFpEF, elevated plasma aldosterone levels have been associated with more pronounced concentric LV hypertrophy[85]. A clinical trial in which HFpEF patients were administered spironolactone or placebo for 12 months demonstrated an
improvement of diastolic function (E/E’) on echocardiography, but did not increase maximal exercise capacity (peak VO$_2$)[86]. The relevance of the observed improvement in E/E’ is unknown. A large multicenter, randomized, double-blind trial (TOPCAT) included almost 3500 patients and they were assigned to spironolactone or placebo, with a mean follow-up of 3.3 years[87]. Overall, spironolactone did not significantly reduce the composite primary endpoint of death from cardiovascular causes, aborted cardiac arrest, or hospitalization for HF. However, a subanalysis revealed important regional variation in the study[88]. Patients recruited in Russia and Georgia appeared to have fewer comorbidities and were younger, but had experienced more myocardial infarctions, had more hospitalizations for HF, lower LVEF and higher diastolic blood pressure compared with patients enrolled in American countries. In Russia and Georgia, there were less clinical events and spironolactone was uneffective whereas in America, with a four times higher event rate, spironolactone reduced rates for the primary outcomes cardiovascular death or hospitalization for HF. In conclusion, aldosterone antagonists may have an indication in HFpEF, but the appropriate selection of patients is still a topic for further research.

**The future: targeting inflammation, oxidative stress and cGMP?**

Overweight and obesity induce inflammatory and oxidative stress with reduced signalling via the NO-sGC-cGMP-PKG pathway, associated with titin hypophosphorylation and increased myocardial diastolic stiffness. Weight loss is therefore expected to yield beneficial effects. Specific effects of weight loss on the heart include decreased LV mass and improved diastolic function[89–91]. As a preclinical demonstration of the beneficial effects of improving NO-based signalling via weight loss, an obese rat model for erectile dysfunction (where the same sGC-cGMP pathway is affected) demonstrated increased NOS expression and lower oxidative stress following bariatric surgery[92]. Although weight loss is often difficult to realize in clinical practice, even in older (>65 years of age) obese adults, lifestyle interventions with caloric restriction and exercise were associated with decreased concentrations of inflammatory molecules and metabolic markers, together with improved muscle quality and physical function[93]. However, larger studies with longer follow-up are needed to evaluate potential effects on the prevention or treatment of HFpEF. Also, it can be considered common sense to treat the underlying cause of the disease before any further treatment can be expected to have optimal effects, in line with treating HFrEF caused by ischemia,
Figure 3. cGMP-PKG pathway modulation. In the normal situation, NO is generated from L-arginine by eNOS. NO stimulates sGC to catalyze the formation of cGMP from GTP. However, cGMP can also be generated by stimulation of pGC by NPs. cGMP exerts its effects viaPKG and both have diverse beneficial cardiovascular effects (green). In the situation of inflammation and/or increased oxidative stress, NO reacts rapidly with $O_2^-$ to form the toxic ONOO$^-$ with many deleterious cardiovascular effects (red). Moreover, inflammation and oxidative stress uncouple the NO producing eNOS dimer into $O_2^-$ generating monomers, further exacerbating oxidative stress. Another effect of oxidative stress and ONOO$^-$ is oxidation of sGC, which leads to loss of its heme-group, making sGC dysfunctional and unresponsive to NO and blocking cGMP formation. Diverse therapeutic strategies are shown in blue. Nitroxy is better tolerated and more stable than nitrates and stimulates sGC. pGC can be stimulated by increasing the availability of NPs, which can be achieved with the combined neprilysin/angiotensin receptor blocker LCZ696. sGC in its inactive, NO resistant heme-free form can be targetted by cinaciguat, a sGC activator. Riociguat and vericiguat mimic NO and are able to directly stimulate sGC. Finally, cGMP can be inhibited by blocking PDEs, such as PDE5 and the (probably) myocardial specific PDE9.

Abbreviations: NO, nitric oxide; eNOS, endothelial NO synthase; sGC, soluble guanylate cyclase; cGMP, cyclic guanosine 3',5'-monophosphate; GTP, guanosine 5'-triphosphate; pGC, particulate GC; NP, natriuretic petptide; PKG, protein kinase G; $O_2^-$, superoxide; ONOO$^-$, peroxynitrite; PDE, phosphodiesterase. Adapted and modified with permission from Hobbs and Stasch[61].
tachyarrhythmias or cardiotoxic drugs. In the case of HFP EF, an equivalent approach would be to treat overweight/obesity and related complications and achieve optimal control of glucose levels and blood pressure, as these maintain inflammatory processes and oxidative stress.

The role for exercise training in HFP EF is still not as clearly defined as in HFrEF. A recent meta-analysis demonstrated that exercise training improves cardiorespiratory fitness and quality of life in HFP EF patients without significant changes in systolic or diastolic LV function [94]. However, most studies only used E/A ratio (the ratio of peak early to late diastolic filling velocity) as a parameter to assess diastolic function, whereas one study showed improved exercise capacity and quality of life to be associated with atrial reverse remodeling and decreased E/E’ in HFP EF patients after an exercise training program for 3 months[95]. Although these results are promising, the timing of a training program and the question whether intensity or duration of exercise training is more important still needs to be investigated and is studied an ongoing trial (NCT02078947).

Other therapeutic strategies target one or more steps of HFP EF pathophysiology, some of which will be briefly discussed next. Tetrahydrobiopterin (BH4) is an essential cofactor for eNOS function and prevents generation of superoxide release from uncoupled eNOS[96]. Although experimental data was promising, clinical studies with BH4 supplementation have been limited and have produced disappointing results so far[97,98]. A class of drugs with proven anti-inflammatory effects are statins, which can also limit oxidative stress in the endothelium[99]. In a recent meta-analysis, statin therapy was associated with a trend towards improved survival in HFP EF, but these findings require confirmation in randomized controlled trials[100]. Resveratrol, an antioxidant and constituent of red wine, berries and peanuts, has been studied extensively in animal models in which it decreased vascular inflammation and oxidative stress and improved endothelial function[101]. However, data from clinical studies are still not sufficiently robust to justify supplementation at this time[102].

Besides the previously discussed weight loss and the PDE5 inhibitor sildenafil, other pharmaceuticals are available that increase cGMP content (Figure 3 and Table 1). cGMP concentrations are regulated via the sGC receptor by NO or via the pGC receptor by NPs, as discussed above[58,59]. Stimulation of these receptors increases cGMP-PKG signalling. Whereas tolerance is a general issue for NO donors, nitroxyl (HNO, a reduced form of NO) is both better tolerated and more stable. Indeed, HNO increased cGMP concentrations and had NOX
suppressing and antihypertrophic actions in rat cardiomyocytes[103]. A recent study with a more translational focus demonstrated that HNO can reduce left and right ventricular filling pressures and systemic vascular resistance in diverse animal models and patients with HFrEF[104]. However, effects in HFrEF still need to be determined. Neprilysin is the enzyme responsible for the breakdown of natriuretic peptides and a new compound, LCZ696, is a combined angiotensin II receptor antagonist -neprilysin inhibitor[105]. LCZ696 was recently shown to be superior to enalapril in reducing the risks of death and hospitalization for HF in a large HFrEF population with LVEF ≤ 40%[106]. LCZ696 was well tolerated in a phase II trial in HFrEF and produced lower levels of NT-proBNP, smaller left atrial volumes, improved functional class and glomerular filtration rates compared to valsartan at 12 weeks. These effects were independent of a reduction in systolic blood pressure[107,108]. Whether these outcomes will translate to an improved HFrEF prognosis has to be awaited and is currently studied in a multicenter (NCT01920711).

sGC can also be activated and stimulated directly. Preclinical studies with the direct sGC activator, cinaciguat, unloaded the heart, increased cardiac output and renal blood flow, and preserved glomerular filtration rate and sodium and water excretion without further neurohumoral activation[109]. However, a phase II trial of intravenous administration of cinaciguat was terminated early due to hypotension even at low doses, but also failed to show an effect on dyspnea and cardiac index[110]. sGC can also be directly stimulated via compounds that mimic NO. The concept of direct sGC stimulation is based on its relative resistance to NO in situations of increased oxidative stress[61]. In phase III trials in patients with pulmonary arterial hypertension and chronic thromboembolic PHT, the oral sGC stimulator riociguat significantly improved symptoms, exercise capacity, pulmonary vascular resistance and NT-proBNP levels[111,112]. Riociguat was also well-tolerated in patients with PHT caused by systolic left ventricular dysfunction, and resulted in improved symptoms, cardiac index and pulmonary and systemic vascular resistance[113]. Currently, the oral sGC stimulator vericiguat (BAY 1021189) is in phase II trial to study its pharmacodynamic effects, safety and tolerability at 12 weeks compared with placebo in patients with HFrEF and in patients with HFrEF[114].

In addition to PDE5, PDE9 was recently shown to be expressed in the mammalian heart (including human) and to be upregulated in hypertrophy and HF. PDE9 regulates NP rather than NO-stimulated cGMP in cardiomyocytes and its
inhibition protects against pathological responses to neurohormones in vitro and sustained pressure overload in vivo[115]. PDE9 expression is increased in the LV myocardium of patients with hypertrophy due to AS (pressure overload), and even more so in patients with HFP EF. These data suggest that inhibition of PDE9 activated PKG and might blunt pathological stress responses. Both PDE5 and PDE9 regulate cGMP-PKG activity, and in combination they could be beneficial in the treatment of HFP EF and thus need to be considered for future research.

CONCLUSION

Metabolic comorbidities are highly prevalent in HFP EF and are probably the driving force in the underlying pathophysiology, causing a chronic, low-grade endothelial inflammation that generates oxidative stress. As a consequence of oxidative stress NO bioavailability is reduced, leading to a subsequent downregulation of the cGMP-PKG pathway and titin hypophosphorylation (a known characteristic of increased myocardial passive stiffness). Many promising treatment strategies that could potentially restore this pathway are currently under study or under development. However, since no pharmacological therapy has yet proven successful in HFP EF, in the meantime a top-down approach of stringent control of comorbidities would probably be the most pragmatic strategy.

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