JACC: BASIC TO TRANSLATIONAL SCIENCE
VOL. 2, NO. 6, 2017
ISSN 2452-302X
https://doi.org/10.1016/j.jacbts.2017.07.013

STATE-OF-THE-ART REVIEW

Murine Models of Heart Failure With Preserved Ejection Fraction

A "Fishing Expedition"

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SUMMARY

Heart failure with preserved ejection fraction (HFpEF) is characterized by signs and symptoms of heart failure in the presence of a normal left ventricular ejection fraction. Despite accounting for up to 50% of all clinical presentations of heart failure, the mechanisms implicated in HFpEF are poorly understood, thus precluding effective therapy. The pathophysiological heterogeneity in the HFpEF phenotype also contributes to this disease and likely to the absence of evidence-based therapies. Limited access to human samples and imperfect animal models that completely recapitulate the human HFpEF phenotype have impeded our understanding of the mechanistic underpinnings that exist in this disease. Aging and comorbidities such as atrial fibrillation, hypertension, diabetes and obesity, pulmonary hypertension, and renal dysfunction are highly associated with HFpEF, yet the relationship and contribution between them remains ill-defined. This review discusses some of the distinctive clinical features of HFpEF in association with these comorbidities and highlights the advantages and disadvantage of commonly used murine models used to study the HFpEF phenotype. (J Am Coll Cardiol Basic Trans Science 2017;2:770–89) © 2017 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

UNDERSTANDING HFpEF IN HUMANS: THE REALITY OF HFpEF TODAY, PATHOPHYSIOLOGY, AND DILEMMAS

HEART FAILURE WITH PRESERVED EJECTION FRACTION (HFpEF): THE CLINICAL ENTITY. Heart failure (HF) is characterized by dyspnea at low-normal levels of activity, and fluid and sodium retention. HF involves impaired heart function, and the percent of blood volume ejected with each beat or ejection fraction (EF), has traditionally served as an indicator of pump dysfunction (1). Decades of extensive basic and clinical research has focused on HF that involves impaired left ventricular (LV) systolic function (systolic HF), also now known as HF with reduced ejection fraction (HFrEF). However, nearly one-half of the patients with HF symptoms have a normal/preserved LVEF. Generally, an LVEF ≥50% is used as a threshold for characterizing as preserved LVEF (2,3). At present, evidence from clinical studies supports the dichotomized distinction that HFpEF and HFrEF are fundamentally and diametrically different with regard to pathophysiology and therapeutic response (4,5).

HFpEF VERSUS HFrEF: WHEN NOT ONLY EF MATTERS. HFpEF and HFrEF are fundamentally different

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All authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

Manuscript received May 30, 2017; revised manuscript received July 25, 2017, accepted July 27, 2017.

beyond segregating and categorizing HF patients into more homogenous groups simply by LVEF. However, this compartmentalization not only allows HF patients with comparable hemodynamics, pathophysiologies, and unique patterns of cardiac and cellular remodeling to be clustered together but also identifies treatment responses in those specific groups (5). HFrEF can be regarded as a cardiac-centric syndrome driven by myocardial cell loss and dysfunction with the heart being subjected to higher wall stress as shown by elevated levels of brain natriuretic peptide (BNP). HFpEF, alternatively, is a systemic syndrome characterized by accumulated risk factors and comorbidities and a noncompliant and stiff heart that is exposed to lower wall stress, reflected in lower BNP levels that, although elevated, are not as high as in HFrEF (6–8). Therefore, it appears that HFrEF begins from the heart and leads to peripheral changes, whereas HFpEF starts in the periphery and culminates at the heart (4,9).

SPEAKING THE SAME LANGUAGE: THE CHALLENGE FOR TRANSLATIONAL HFpEF RESEARCHERS. Semantics related to HFpEF are a formidable task for translational researchers. Defining various terms, and therefore collecting relevant data, remains a challenge both clinically and in the preclinical area. One of the main sources of confusion lies in the distinction between diastolic dysfunction and HFpEF. These 2 terms have been used interchangeably in both the preclinical and the clinical literature. Diastolic dysfunction was widely and incorrectly touted to be the sine qua non for HFpEF but by itself is not enough to establish it. For example, normal subjects may have diastolic dysfunction, yet have no clinical features of HFpEF (10,11). Additionally, diastolic dysfunction is a common occurrence in HFrEF (12). Also, use of strain imaging with echocardiography shows subtle abnormalities in systolic function in some HFpEF populations despite preserved global LVEF (13,14). According to the 2013 American College of Cardiology Foundation (ACCF)/American Heart Association (AHA) guideline for the management of HF (3), neither LV hypertrophy nor diastolic dysfunction is required for diagnosis of HFpEF, whereas in the European HF guidelines relevant structural and/or diastolic dysfunction is a prerequisite (2). HFpEF is also a term, in the sphere of scientific literature, that is virtually nonexistent, given that the U.S. National Library of Medicine's system of medical subject headings (MeSH) still uses the outmoded terms "HF, systolic" and "HF, diastolic" to index publications. Hence, we prefer the terms HFpEF and HFrEF as they are mutually exclusive, whereas diastolic dysfunction may be present in either systolic HF (HFrEF) or diastolic HF (HFpEF). However, we acknowledge that until universally agreed definitions are adopted, translational research in the field may be hindered.

MODELING HFpEF IN THE LAB: DIFFERENT PHENOTYPES FOR A COMPLEX DISEASE

Animal models, as opposed to isolated organ and/or cell preparations, allow examination of physiological effects of cardiac function (15). However, the search for an animal model that resembles the human HFpEF phenotype is akin to "a fishing expedition" and the use and comprehensive characterization of an HFpEF animal model that recapitulates human HFpEF has hampered advancing the understanding of HFpEF. The limited number of truly authentic HFpEF animal models poses a major limitation when investigating new insights into its pathophysiology and in the development of new therapies for HFpEF (16,17). Moreover, if defining HFpEF in humans evokes controversy (18), it appears that animal models likely follow suit. Animals, unlike humans, cannot report symptoms. LVEF, diastolic dysfunction, and heart structure can be measured directly, but signs must be inferred from animal behavior. Additionally, separate from LVEF, a single measure of systolic function, there are other measures of LV systolic function such as myocardial velocities, strain, and strain rate (measures of myocardial contractility), which may be impaired in HFpEF (13,14,19). HFpEF is a complex syndrome where etiologic and pathophysiological paths, by which individual patients develop the disease, are variable (20). Specific comorbidities, as well as aging, are highly associated with the HFpEF phenotype. Given this heterogeneity, it is likely that any animal model only resembles a certain proportion of HFpEF patients. An "ideal" animal model should meet various requirements to mimic human disease, including cardiac, hemodynamic, neurohormonal, and peripheral aberrations commonly seen in HFpEF patients (21). However, similar to humans, a "one-size-fits-all" strategy is unlikely to work in animal models. That being said, the relative importance of comorbidities on the initiation, development, and treatment of HFpEF is unknown (22). A more tailored approach focusing on specific phenotypes is needed in the laboratory to understand the complex interactions underlying this disease. HFpEF in humans

ABBREVIATIONS AND ACRONYMS

AAC = ascending aortic constriction

BNP = brain natriuretic peptide

DOCA = deoxycorticosterone acetate

EF = ejection fraction

HF = heart failure

HFpEF = heart failure with preserved ejection fraction

HFrEF = heart failure with reduced ejection fraction

LV = left ventricular

NaCl = sodium chloride

SAMP = spontaneous senescence prone

SAMR = spontaneous senescence resistant

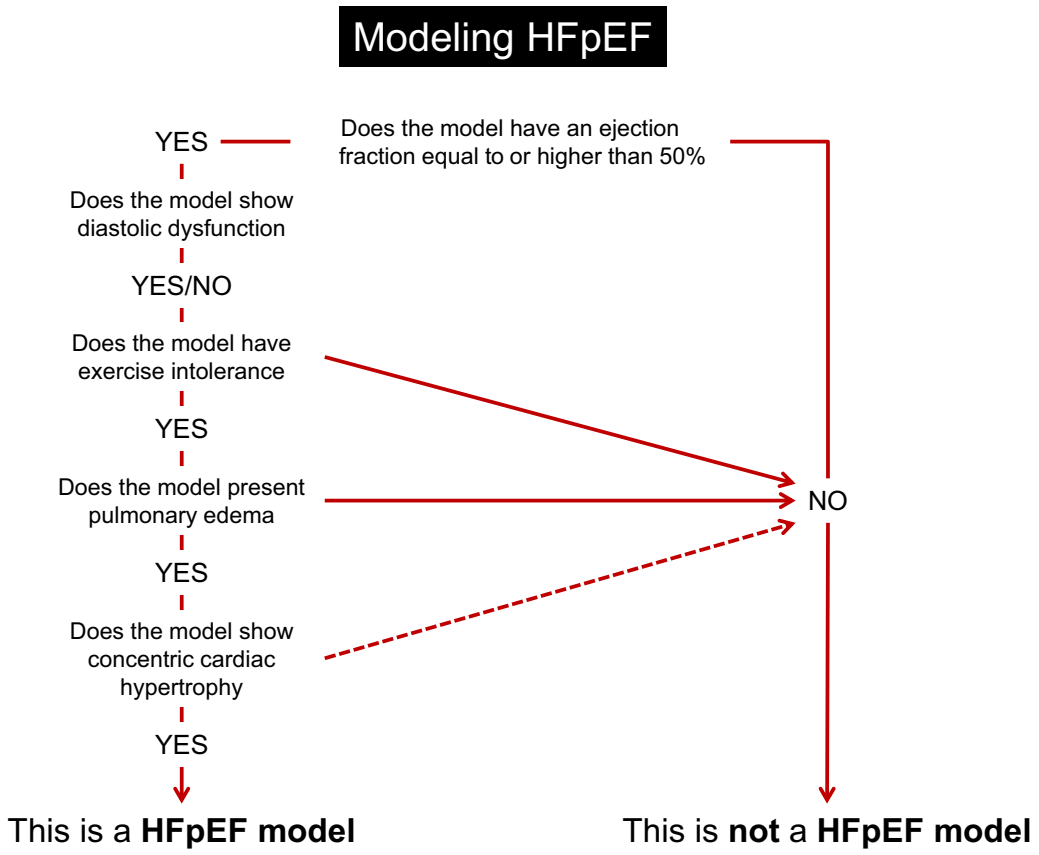
SHR = spontaneously hypertensive rat

TAC = transverse aortic constriction

ZSF1 = Zucker fatty and spontaneously hypertensive heart failure rat

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 2, NO. 6, 2017
DECEMBER 2017:770–89

FIGURE 1 Flowchart Identifying Major Features to Fulfill When Modeling HFpEF in Preclinical Studies



is strongly associated with diseases such as hypertension, obesity, and diabetes mellitus (3,6), which by themselves often occur together as part of the metabolic syndrome. Yet there is much overlap between these comorbidities, and a direct causal relationship between one and the other and HFpEF has not been established. However, if such a causal relationship can be elucidated, a step toward developing targeted therapies might be in sight. A major benefit of an animal model is that it is easier to examine the contribution of each risk factor in isolation without additional comorbidities confounding the findings. Animal models are useful to understand the effect of interventions on the disease process, which in turn helps in the development of therapeutic approaches. Murine models are convenient and inexpensive, particularly in the quest to investigate the molecular basis underlying HFpEF. This is due to the availability of genetically engineered strains and molecular techniques to manipulate sub cellular processes occurring in the heart, as well as the relatively short life span of murine versus larger animal models (23,24). It is important to stress that although murine models offer easy access to genetic manipulations, extensive characterization of the actual HFpEF phenotype is required to be fully useful for HFpEF studies (25). This being said, specific genetic manipulations may be useful for the development of therapeutics by investigating the specific role of a molecule/factor in the HFpEF syndrome. However, in contrast, specific genetic manipulations that manifest a purported HFpEF phenotype are of little use in understanding common forms of HFpEF or as a disease model.

In this review, we consider whether some of the available murine models of cardiac hypertrophy and HF recapitulate human HFpEF disease. Attention has been paid to those phenotypical characteristics that would characterize an “ideal” HFpEF animal model, which are included in **Figure 1**. Although diastolic dysfunction is certainly part of the compendium of the HFpEF phenotype, it is only 1 piece of the puzzle. It is also necessary to identify models with systemic alterations that are implicated in human HFpEF. These include impairments in peripheral function, such as exercise intolerance, alterations in pulmonary

TABLE 1 Murine Models of HFpEF Classified by Aging and Comorbidities Seen in HFpEF Patients (Hypertension, Obesity and Diabetes, Atrial Fibrillation, Pulmonary Hypertension, and Renal Insufficiency)

		HFpEF Pathophysiology						
Comorbidity	Animal Models	LV Structure	Systolic Function	Diastolic Function	Pulmonary Congestion	Exercise Intolerance	HFpEF	Ref. #
Aging	Fisher 344 rat	Eccentric hypertrophy	Mild dysfunction	Diastolic dysfunction	Yes	Yes	☹	(33,38–40,42,43)
	SAMP8 mouse	Not described	Preserved to reduced	Diastolic dysfunction	No	Yes	☹	(49–51)
Hypertension	Aldosterone-infused uninephrectomized mouse	Concentric hypertrophy	Preserved	Diastolic dysfunction	Yes	Yes	☺	(68,70–74)
	Angiotensin II-infused mouse	Concentric and dilated hypertrophy	Preserved	Diastolic dysfunction	Yes	Yes	☺	(16,81,82,84–86,89–91)
	Dahl salt-sensitive rat	Concentric to eccentric hypertrophy	Preserved to reduced	Diastolic dysfunction	Yes	Yes	☹	(95–98)
	DOCA salt rat	Concentric hypertrophy	Preserved	Diastolic dysfunction	Not described	Not described	?	(107,108)
	Spontaneously hypertensive rat	Concentric to eccentric hypertrophy	Preserved to reduced	Diastolic dysfunction	Yes	Yes	☹	(119–122)
	Transverse aortic constriction-induced pressure overload mouse	Concentric to eccentric hypertrophy	Preserved to reduced	Diastolic dysfunction	Yes	Yes	☹	(70,127–129)
Obesity and diabetes	db/db mouse	Concentric hypertrophy	Preserved	Diastolic dysfunction	Yes	Yes	☺	(152,155,159–165)
	ob/ob mouse	Concentric hypertrophy	Preserved	Diastolic dysfunction	Not described	+/−	?	(175–178,185)
	Streptozotocin-induced diabetic rat	Eccentric hypertrophy or heart atrophy	+/−	Diastolic dysfunction	+/−	Not described	☹	(191,195–197,200,203,204,207,208,212,213)
	ZSF1rat	Concentric hypertrophy	Preserved	Diastolic dysfunction	Yes	Yes	☺	(226,228,229,231)
Atrial fibrillation	Genetic model	None or eccentric hypertrophy	Mild dysfunction	Not described	Not described	Not described	?	(245)
Pulmonary hypertension	Hypoxia-induced pulmonary hypertension	Concentric hypertrophy	Preserved	Not described	Not described	Not described	?	(267,268)
Renal insufficiency	Subtotal nephrectomy rat	Concentric to eccentric hypertrophy	Preserved to reduced	Diastolic dysfunction	Yes	Yes	☹	(275,277–279,281,282)

☺ = HFpEF phenotype; ☹ = not a HFpEF phenotype; HFpEF = heart failure with preserved ejection fraction.

physiology, and cardiac morphology (such as hypertrophy, fibrosis, and capillary rarefaction). We chose to emphasize models that induce HFpEF by the most common comorbidities associated with human HFpEF, although we acknowledge that murine models are less influenced by comorbidities than patients. Table 1 outlines the models included in the present review, divided into aging and comorbidities seen in HFpEF patients (hypertension, obesity and diabetes, atrial fibrillation, pulmonary hypertension, and renal dysfunction).

AGING HFpEF MODELS. According to the Framingham Heart Study and the Baltimore Longitudinal Study on Aging, there is an increased prevalence of LV hypertrophy with age, even in the absence of clinical hypertension. Diastolic function worsens in the elderly, whereas LVEF is relatively preserved in subjects at rest (26–28). Aging primarily plays a role in modifying both the passive stiffness of the myocardium and the active diastolic relaxation properties of the cardiomyocyte (28). Alterations in the peripheral vasculature, pulmonary function, oxygen transport, and skeletal muscle function, during aging are also key determinants of exertional fatigue and/or dyspnea in human HFpEF (29).

Mice and rats are commonly used to study aging and age-related diseases (30). Their relatively short life span makes them easier and more cost-effective to study than long-lived animals. Cardiac aging in murine recapitulates many changes observed in humans, with age-dependent increases in LV

hypertrophy as well as impaired diastolic function (31,32). While systolic function declines only slightly with age, the maximal exercise capacity and O_2 consumption are significantly reduced (28). Likewise, progressive myocardial degeneration and fibrosis are known to develop in rats with aging (33). Mechanisms implicated in age-associated changes in myocardial structure and function include cardiomyocyte enlargement (34), decrease in cardiomyocyte number due to increased apoptosis and necrosis (35,36), and compensatory cardiac remodeling with alterations in extracellular matrix composition and cardiac fibroblast number and function (26).

Fischer 344 rat. The Fisher 344 aging rat mirrors the pathophysiology of aging in various organs, including the heart (17). By 28 months to 30 months of age, these rats have cardiac hypertrophy comparable to rats subjected to long-term ascending aortic constriction-induced pressure overload. This cardiac hypertrophy is accompanied by LV chamber dilation, infrequently seen in HFpEF (37), and by extensive interstitial fibrosis with subsequent loss of ventricular cardiomyocytes by 36 months (33,38). Between 22 months and 30 months of age, the Fischer 344 rat develops mild, progressive LV diastolic dysfunction, with impaired LV relaxation and decreased LV compliance (33,39–42). Exercise intolerance is also apparent (43). Pulmonary congestion is evident at 25 months of age, with a modest decline in LV systolic function (43). Although a priori, signs of HF are evident; the development of eccentric hypertrophy and LV dilation makes the Fisher 344 rat limited as an HFpEF model.

Spontaneous senescence prone (SAMP) mouse. The SAMP mouse is a model of spontaneous senescence that shows disorders commonly seen in aging humans, such as neurodegeneration or carcinogenesis (44–46). The senescence-prone (SAMP) and control senescence-resistant (SAMR) strains were singled out by continuous sister-brother mating (47). The SAMR strains show normal aging characteristics, whereas the SAMP strains show accelerated senescence and age-related pathological phenotypes, similar to aging disorders seen in humans. The aging features in SAMP mice occur at a younger age (10 months to 14 months) compared with normal-aged mice (≥20 months) (48). SAMP8 and SAMR1 are the best-studied strains with respect to cardiovascular disease (49). SAMP8 mice show cardiac dysfunction and adverse cardiac remodeling during senescence (50) with evidence of cardiomyocyte hypertrophy and fibrosis (49,51). These mice also develop diastolic dysfunction independent of blood pressure changes (49). Systolic function is similar (49) or slightly decreased (51) in SAMP8 compared with SAMR1, but LVEF appears preserved. Evidence suggests that this is a model of diastolic dysfunction; however, no studies have described the presence of HF or HFpEF.

HYPERTENSION-INDUCED HFpEF MODELS. Systemic hypertension is the single most important comorbidity seen in HFpEF, with a prevalence of 60% to 89% reported from large controlled trials, epidemiological studies, and HF registries (3). Elevated blood pressure is a major determinant of LV structural alterations. The relationship between systemic hypertension and LV hypertrophy is well known, with elevated blood pressure in midlife correlating with LV hypertrophy in later life (52). Increased blood pressure induces cardiomyocyte and fibroblast changes and accelerates cardiac remodeling. Moreover, hypertension results in vascular changes such as endothelial dysfunction, reduced coronary reserve blood flow, and diminished capillary density, all of which lead to reduced oxygen delivery. Systemic hypertension also results in arterial stiffness, which imposes a disproportionate load on the heart, leading to ventricular-vascular uncoupling and afterload mismatch (53). These changes lead to impaired systolic and diastolic function (15). Although blood pressure reduction in patients with diastolic dysfunction is an effective approach to improving echocardiographic parameters (54,55), there is no clear evidence that blood pressure reduction in HFpEF improves outcomes (56). Additionally, although hypertension is a major risk factor for HFpEF, "management" of hypertension (which encompasses diagnosis and blood pressure treatment and control) has not decreased the incidence or prevalence of HFpEF (57–61).

Murine models of hypertensive HFpEF have many of the features of human HFpEF, as shown in **Table 2**. There are a wide range of models, and most mimic some aspects of the relevant human HFpEF disease, but no single model recapitulates all, although some more than others. A major deficiency in these models is that they do not reproduce the slow onset seen in patients with hypertension that lead to HFpEF development (62).

Aldosterone-infused and unilateral nephrectomized mouse. Pioneering studies more than 2 decades ago reported that the combination of uninephrectomy, aldosterone infusion, and 1% sodium chloride (NaCl) administration in rats induced blood pressure elevation, cardiac hypertrophy, and fibrosis (63–65). This effect was also described in mice (66). Circulating aldosterone levels in this HFpEF model are ~6.0 to 7.5 ng/ml (67,68), comparable to circulating levels of aldosterone seen in human acute HF (mean of 18 ng/ml) (69). Mice

TABLE 2 Murine Models: Pathophysiology of Hypertension-Associated HFpEF Based on the Existing Literature

Findings in Hypertension-Associated Human HFpEF (15)	Aldosterone-Infused Mouse	Angiotensin II-Infused Mouse	Dahl Salt-Sensitive Rat	DOCA Salt Rat	Transverse Aortic Constriction Mouse
Elevated blood pressure	Yes	Yes	Yes	Yes	Yes
Fatigue/exercise intolerance	Yes	N/A	Yes	N/A	Yes
Pulmonary congestion	Yes	N/A	Yes	N/A	Yes
Concentric hypertrophy or increased LV mass	Yes	Yes	Yes	Yes	Yes
Impaired active relaxation	Yes	Yes	Yes	Yes	Yes
Impaired passive filling	Yes	Yes	Yes	Yes	Yes
Enlarged left atrium	N/A	N/A	Yes	N/A	Yes
Cardiomyocyte hypertrophy	Yes	Yes	Yes	Yes	Yes
Myocardial fibrosis	Yes	Yes	Yes	Yes	Yes
Decreased intra-myocardial capillary density	N/A	Yes	Yes	Yes	N/A
Increased biomarkers such as NT-proBNP, BNP, and troponin	Yes	Yes	Yes	Yes	Yes

BNP = brain natriuretic peptide; LV = left ventricular; N/A = not available; NT-proBNP = N-terminal pro-brain natriuretic peptide; other abbreviation as in Table 1.

subjected to uninephrectomy and aldosterone infusion for 4 weeks, accompanied by 1% NaCl intake, develop HFpEF with moderate hypertension, concentric LV hypertrophy, pulmonary congestion, and echocardiographic evidence of diastolic dysfunction while maintaining a normal/preserved LVEF (68,70,71). These mice also show exercise impairment (72). At the molecular level, LV tissue from these mice show an increase in natriuretic peptides, cardiac size, (73), as well as an increase in the expression of the titin transcript variants n2ba and n2b (74). Titin plays a pivotal role in diastolic stiffness in HFpEF (75,76) along with the extracellular matrix (77). In addition to increased myocardial oxidative stress, there is altered expression of cardiomyocyte proteins that are involved in the mobilization of calcium during the excitation-contraction coupling process in the heart. Sarcoplasmic reticulum Ca(2+)-adenosine triphosphatase expression is decreased, as are protein kinase A (PKA)-dependent and Ca2+/calmodulin-dependent protein kinase II (CAMKII)-dependent phosphorylation of phospholamban (68). Interestingly, in this model, serum creatinine is mildly increased, along with an increase in glomerular size and urine albuminuria, indicating impaired renal function (67), which is seen in human HFpEF. Thus, this model recapitulates the clinical HFpEF phenotype and also features molecular changes that have been described clinically (75,78–80).

Angiotensin II–infused mouse. Administration of angiotensin II for a variable timeframe (1 to 8 weeks) in mice leads to cardiac hypertrophy and remodeling, both in the presence (81–85) and absence (16,86) of hypertension, suggesting that cardiac remodeling under angiotensin II infusion is due to blood pressure-dependent and -independent factors. It is apparent that the observed elevation in blood pressure is dependent on the dose of angiotensin II used. However, the relevance of supraphysiological circulating angiotensin II levels in human disease has been questioned. In addition, although it is difficult to extrapolate from studies in different species, any potential comparison is complicated by the fact that the majority of angiotensin II-infusion animal studies have not measured or do not report circulating blood levels (87). Moreover, cardiac hypertrophy appears strain-specific. Whereas C57BL/6J mice develop compensatory concentric hypertrophy and fibrosis in response to angiotensin II (88), Balb/c mice show severe LV chamber dilatation (89), which is infrequently seen in HFpEF and more characteristic of a dilated cardiomyopathy. Although, the angiotensin II-infused mouse model demonstrates diastolic dysfunction (81,84,90,91), it has shown both a lack of change in systolic function (16,86) as well as a decrease in LVEF (92) and these discrepant findings are likely dose-dependent. Pulmonary congestion (89), as well as exercise intolerance, are evident and seem to be related to angiotensin II-induced skeletal muscle abnormalities, including impaired mitochondrial function and skeletal muscle atrophy (93). In summary, if strain and dosage are optimized to mirror the human HFpEF phenotype, angiotensin II infusion appears to be a relevant HFpEF model. This corroborates, once again, the importance of the experimental design when performing preclinical studies in the field.

Dahl salt-sensitive rat. This mutant strain of the Sprague-Dawley rat is characterized by increased salt sensitivity and is only genetically distinguishable from the Dahl salt-resistant rat by a polymorphism in the renin gene (94). When placed on a high-salt diet (8%) at 6 weeks to 8 weeks of age, the Dahl salt-sensitive rats develop a steep elevation in blood pressure and progressive concentric LV hypertrophy, precipitating HFpEF at approximately 14 to 19 weeks

(95-97). Signs of HF include tachypnea, labored breathing, inactivity, and postmortem evidence of pulmonary edema and ascites (95,98). However, with continued exposure to a high-salt diet (~26 weeks) or when diet is introduced at an older age, these animals develop HFrEF (23,95). Thus, this model is used to study the transition from compensated cardiac hypertrophy to HFrEF (23,95), something uncommonly seen in human HFpEF (99,100). Dahl salt-sensitive rats also have renal dysfunction and metabolic disturbances, including insulin resistance and dyslipidemia (17). A relative strength of the Dahl salt-sensitive rat is that its cardiac phenotype is due to salt and water retention, quite reflective of the clinical situation (101,102). The demographics and comorbidities commonly found in HFpEF are strongly associated with blood pressure salt sensitivity. Similarly, sodium restriction in salt-sensitive hypertensive HFpEF patients reduces systemic blood pressure, arterial stiffness, and oxidative stress (102). However, this model lacks clinical applicability as an HFpEF model because very high systolic blood pressure values (>175 mm Hg) develop (95,103), compared with humans, where often only moderate hypertension is seen (15). In addition, this HFpEF model will progress to HFrEF with continued salt administration.

Deoxycorticosterone acetate (DOCA)-salt rat and mouse. The DOCA-salt hypertensive rat model was first described in 1969 (104). DOCA is administrated by intraperitoneal injections or subcutaneous pellet implantation for 4 weeks, accompanied by unilateral nephrectomy (1 week before DOCA, at 6 to 10 weeks of age) and 1% NaCl drinking water (105,106). This combination of procedures causes hypertension, cardiac hypertrophy, and perivascular fibrosis (107). Information regarding HFpEF development in this model is limited, but concentric hypertrophy accompanied by severe restrictive diastolic dysfunction in the presence of a preserved LVEF occurs 28 days after DOCA (108). More recently, DOCA has been used in mice. The DOCA-salt mouse develops cardiac hypertrophy, whereas blood pressure is either unchanged or only mildly increased (109,110). Thus, it is unclear whether it is a hypertension-HFpEF model. Cardiac hypertrophy associates with increased fibrosis and vascular rarefaction (110). Increased myocardial oxidative stress, nitric oxide synthase-dependent superoxide production, and reduced nitric oxide production are also seen (111). DOCA-salt mice show diastolic dysfunction with preserved systolic function (109,111,112) accompanied by exercise intolerance, but without pulmonary congestion or HF (113).

Spontaneously hypertensive rat (SHR). This inbred strain is pre-disposed to hypertension, with evidence of pre-hypertensive, developing, and sustained phases, similar to essential hypertension in humans (114-116). The SHR cardiovascular literature is vast, and its resemblance to the human condition and the relative lack of individual variation in this strain makes this an attractive model (17,117). It develops compensated concentric LV hypertrophy while preserving contractility during the first year of age but thereafter develops overt HFrEF characterized by LV dilatation with diastolic dysfunction (118-120) which is uncommonly seen in human HFpEF (99,100). By 18 months to 24 months of age, more than one-half of the SHRs develop cardiac decompensation, with tachypnea and severely decreased LVEF (121). However, some colonies of SHR lack this decline in LVEF at 20 months, despite the evidence of diastolic dysfunction, concentric remodeling, and lung congestion (122). Abnormalities in myocardial mechanics, such as strain and myocardial velocities, occur together with T-tubule disorganization and impaired Ca(2+) cycling in the hypertensive SHR, which occur before the development of cardiac fibrosis, cardiac dysfunction, and HF (19). Although HF in SHR occurs around 2 years of age, mimicking some aspects of the human disease where HFpEF is highly prevalent in the aging population (21), this is a time-consuming and therefore expensive model. Importantly, despite the lengthy duration required for HF development, data regarding the presence of preserved LVEF in this model are limited and confusing, making it less attractive for HFpEF research. Basic researchers should systematically determine which specific SHR colonies are more likely to develop HFpEF and use those animals for study. Importantly, the transition in SHR from compensated cardiac hypertrophy to HFrEF failure limits its use as an HFpEF model. As discussed earlier, the transition from HFpEF to HFrEF (by dropping LVEF) is uncommon in human HFpEF.

Thoracic aortic constriction-induced pressure overload in mouse. Aortic constriction is a well-established surgical procedure for the induction of LV chronic pressure overload. It was first described in 1991 (123) and since then has been widely used to investigate cardiac hypertrophy. In this model, a band is placed around the aorta of a young mouse and, as the mouse grows, the band becomes obstructive, resulting in cardiac hypertrophy, with increased cardiomyocyte size and collagen volume fraction (124,125). Thoracic aortic constriction can either be close to the origin of the aorta (ascending aortic constriction, AAC) or in the aortic arch between the brachiocephalic and the left carotid arteries

(transverse aortic constriction, TAC) (23). AAC is technically more challenging and generally used to study acute effects of early pressure overload, independent of hypertension, because mice do not show an increase in blood pressure. Moreover, diastolic dysfunction is secondary to pressure overload and dependent on the severity and the rapid onset of the constriction. As such, AAC represents more closely a model of aortic valve stenosis rather than a model of HFpEF, where a clinical scenario of acutely increased afterload is not commonly observed (16,17); that is, aortic stenosis is an uncommon cause of HFpEF and may proceed directly to HFrEF. In contrast, TAC results in a slightly more gradual rise of LV pressure and progression toward cardiac hypertrophy and HF (23,126). In the preclinical literature, TAC is generally used as a model of diastolic dysfunction. Both AAC and TAC are characterized by an initial compensatory phase, with concentric LV hypertrophy, followed by cardiac chamber enlargement and eccentric LV remodeling, resulting in further deterioration of the LV systolic function, including a decline in LVEF (126). There is procedural variability depending on the severity of constriction of the banding, the age of the mouse at the time of the surgery, and, more importantly, the length of follow-up. In general, AAC and TAC both result in an acute imposition of LV pressure load. Thus, signs of HF, including LV concentric hypertrophy, diastolic dysfunction, and lung congestion are evident after 2 to 3 weeks of constriction (70,127). It then progresses to HFrEF, with LV chamber dilatation and impaired systolic function after ≥4 weeks (128,129). However, the progression from HFpEF to HFrEF is atypical in humans (99,100,103). In response to pressure overload, the heart undergoes remodeling at the structural, cellular, and molecular level. The hallmark of this remodeling includes increased collagen deposition resulting in a stiff myocardium and recapitulation of the fetal gene program such as increased expression of the β-isoform of myosin heavy chain (130). Moreover, similar to the aldosterone-infused model, pressure overload decreases sarcoplasmic reticulum Ca(2+)-adenosine triphosphatase expression and downregulation of phospholamban expression as early as 2 weeks post-TAC (131).

METABOLIC PHENOTYPE: OBESITY AND DIABETES MODELS. Large outcome trials and registries reveal that being overweight or obese is a major risk factor for HFpEF (6,132). Obesity induces significant structural changes in the LV (133), and patients with HFpEF are significantly more likely to be obese. A body mass index >35 kg/m^2 is associated with increased mortality and hospitalization rates (134). There are multiple mechanisms whereby obesity could contribute to HFpEF. Increased adiposity promotes inflammation, insulin resistance, and dyslipidemia and also impairs arterial, skeletal muscle, and physical function (135–138), all of which are abnormal in patients with HFpEF (1,9,135,139). Until recently, sparse attention has been paid to increased adiposity and obesity in HFpEF (139), with no clinical trials addressing the role of obesity in HFpEF. Weight loss after bariatric surgery is reported to decrease cardiac hypertrophy and LV filling pressures and to improve diastolic dysfunction (140). However, this strategy has not been specifically studied in the HFpEF population (141,142).

Diabetes is also commonly seen in HFpEF (143). Although its presence is associated with a poor prognosis in HF, regardless of LVEF, the relative risk of cardiovascular death or HF hospitalization conferred by diabetes is greater in HFpEF than in HFrEF (144). There are multiple mechanisms by which diabetes could perpetuate HFpEF. Systemic insulin resistance and hyperglycemia trigger cardiac insulin resistance and neurohormonal, sympathetic, and cytokine imbalance in the heart (145,146). This, in turn, might induce cardiac remodeling processes such as cardiomyocyte hypertrophy, interstitial fibrosis, and collagen and titin modifications, leading to further cell damage and deterioration of diastolic and systolic function (147,148). Peripheral mechanisms may also mediate the deleterious effect of diabetes in HFpEF. Prolonged hyperglycemia and hypo- or hyperinsulinemia contribute to skeletal muscle dysfunction with changes in glucose use and capillary density (149). These may all limit exercise capacity, one of the earliest symptoms of HFpEF.

Murine models of obesity and diabetes-induced HFpEF have several limitations compared with the human phenotype. First, preclinical models usually present with fulminant and uncontrolled hyperglycemia or insulin resistance, which does not mimic the clinical situation in HFpEF (150). Studies performed before the onset of diabetes most likely reflect changes due to obesity and insulin resistance, and studies performed after the onset of diabetes reflect the added effects of hyperglycemia of variable duration because insulin resistance and diabetes develop at different stages (23). Lastly, although several models showed the effect of diabetes per se, in the absence of obesity, on the onset of HFpEF, there is sparse information regarding an obesity model alone that does not have insulin resistance or diabetes (which are cofounders in an obesity model), which therefore limits investigating the exclusive role of

TABLE 3 Murine Models: Pathophysiology of Obesity and Diabetes-Associated HFpEF Based on the Existing Literature

Findings in Obesity and Diabetes-Associated Human HFpEF (53,139)	db/db Mouse	ob/ob Mouse	Streptozotocin-Induced Diabetic Rat	ZFS1 Rat
Obesity	Yes	Yes	No	Yes
Insulin resistance or diabetes	Yes	Yes	Yes	Yes
Fatigue/exercise intolerance	Yes	+/−	N/A	Yes
Edema	Yes	N/A	+/−	Yes
Concentric hypertrophy or increased LV mass	Yes	Yes	+/−	Yes
Arterial stiffness	Yes	Yes	Yes	Yes
Disturbed ventricular-arterial coupling	Yes	N/A	Yes	N/A
Cardiomyocyte hypertrophy	Yes	Yes	Yes	Yes
Myocardial fibrosis	Yes	Yes	Yes	Yes
Decreased intra-myocardial capillary density	Yes	+/−	Yes	No
Natriuretic peptides levels	↓	=/↓	↑	N/A
Systemic inflammation	Yes	N/A	Yes	N/A

Abbreviations as in Tables 1 and 2.

increased adiposity in HFpEF. Table 3 shows the major HFpEF features seen in the following murine models.

db/db mouse. The db/db leptin receptor-deficient mouse has a point mutation in the diabetes (db) gene encoding the leptin receptor, which spontaneously causes morbid obesity accompanied by severe hyperglycemia secondary to type 2 diabetes (151,152). The progression of diabetes in the hyperleptinemic db/db mice, with initial insulin resistance followed by an insulin secretion defect, is similar to the pathogenesis of type 2 diabetes in humans (153). Thus, this model is valuable in exploring the combined contribution of obesity and type 2 diabetes to HFpEF, which is representative of this particular HFpEF phenotype (16). db/db mice show an inflammatory, systemic cytokine fingerprint characterized by increased proinflammatory cytokines in early disease (8 weeks) but lower levels of a similar proinflammatory profile at older ages (28 weeks) (154). Despite the presence of both hyperinsulinemia and hyperleptinemia, mice do not initially show cardiac hypertrophy (152,155-157), but it eventually develops at older ages (158-160). At the histological level, these mice hearts have enlarged cardiomyocytes (158), evidence of fibrosis (161), and capillary rarefaction. There is also diastolic dysfunction with increasing age, whereas systolic function remains preserved even at 6 months of age (152,158,162,163). Diabetic db/db mice show abnormal ventricular-arterial coupling due to decreased vascular compliance and increased LV stiffness. The latter is associated with increased titin-isoform N2B expression (152). Exercise intolerance parallels diastolic dysfunction and is severely reduced by 12 weeks of age (164), with evidence of pulmonary congestion (165). Natriuretic peptide levels are also reduced (166), similar to the human obesity phenotype. Obese HF patients often have low circulating natriuretic peptides levels for a given degree of HF (167). In conclusion, the db/db mice appear to represent the obese/metabolic HFpEF phenotype, with evidence of HF, whereas LVEF is preserved. Importantly, the reduced levels of natriuretic peptides make this model even more attractive because this is a specific feature seen in obese HFpEF patients.

ob/ob mouse. The ob/ob leptin-deficient mouse spontaneously develops obesity and type 2 diabetes secondary to hyperphagia, hyperglycemia, and hyperinsulinemia (168,169). These mice are indistinguishable from their lean littermates (ob/+) at birth, but within 2 weeks gain weight and are hyperinsulinemic. By 4 weeks of age there is also marked hyperglycemia. Blood glucose rises to reach a peak after 3 to 5 months, accompanied by accelerated food intake and rapid growth (169-172). Thereafter, it decreases and eventually normalizes in older mice, although they remain insulin resistant (170,173,174). These mice develop myocardial hypertrophy, with increased cardiomyocyte size (175-177) in parallel with diastolic dysfunction, but without changes in systolic function (178). Compared with ob/+ mice, variable arterial blood pressure is observed (176,179,180). There is also cardiac fibrosis (181) and increased arterial stiffness due to a destabilized elastic fiber network and increased elastolytic activity (182). Natriuretic peptide cardiac expression is reportedly unchanged or slightly reduced compared with control ob/+ mice (178,181,183,184). Moreover, although ob/ob mice are less active than their control, this is attributed to excess body weight rather than to their cardiac phenotype (185). Survival is decreased in ob/ob mice compared with ob/+ mice (186), and it appears that they die before cardiac hypertrophy progresses to overt HF (177). The main disadvantages of this model are 2-fold. First, at least some of the cardiac changes observed appear to be secondary to the loss of leptin-mediated signaling (187). The aberrations seen in ventricular cardiomyocytes from ob/ob mice, such as cellular hypertrophy, depressed peak cell shortening, reduced maximal velocities of shortening/re-lengthening, and prolonged duration of re-lengthening, are reversed by recombinant leptin treatment (158,176). Additionally, obese humans with leptin deficiency are uncommon, so the ob/ob mice do not mimic the human phenotype (169,188).

Streptozotocin-induced diabetic rat. Streptozotocin administration in rats was first described in 1963 (189). Rapid β-cell degranulation and necrosis occurs

7 to 10 h after injection, and causes hyperinsulinemia and hypoglycemia. After longer periods (28 days), prolonged hyperglycemia and a reduction in pancreatic insulin levels ensues (190). Streptozotocin induces either type 1 or type 2 diabetes, depending on the dosage and duration of administration. Doses of 50 to 65 mg/kg lead to hyperglycemia without ketosis, and insulin administration is not required. However, at higher doses (75 mg/kg and greater) spontaneous ketosis and death occurs within days if insulin is not administered (191). Streptozotocin-injected rats exhibit many of the cardiovascular complications found in humans with both type 1 and type 2 diabetes. They have increased levels of cardiac and circulating natriuretic peptides (192,193) and inflammatory markers, such as interleukin 1, interleukin 6, and intracellular adhesion molecule 1 (194). Blood pressure remains within normal ranges, indicating that hyperglycemia alone may be sufficient to account for the observed changes seen in the heart in this model (17). This includes eccentric cardiac hypertrophy, gradual diastolic dysfunction with impaired contractile performance, and increased ventricular stiffness (191,195–197). Fibrosis and a titin-isoform pattern switch is observed (198–200), correlating with increased ventricular stiffness (201). Streptozotocin-induced diabetic rats also show altered systolic load of the left ventricle coupled to the arterial system and decreased systemic arterial compliance (202). Although relative lung weights are reported to be increased (203), this is more likely due to a decrease in body weight than to the presence of lung congestion per se, as other studies showed no differences in lung weights relative to tibia length or wet-to-dry lung ratio (204,205). Cardiac atrophy is also described, with chronic apoptotic cardiomyocyte loss and inadequate reactive hypertrophy (206–208). There is also significantly reduced cardiac capillary density (209). A major disadvantage of using the streptozotocin-induced diabetes model in cardiovascular and HFpEF studies is that some of the purported cardiac effects are partly attributed to the direct toxic actions of streptozotocin itself (210). More importantly, LVEF values in this model are variable, with some studies showing a preserved LVEF (200,211–213), whereas others show a reduction in LVEF during diabetes development (214). This discrepancy could be due to the severity of diabetes or the methods used to evaluate cardiac function (215).

Zucker rat. In 1961, a mutation designated “fatty” appeared spontaneously in a group of outbred rats giving rise to the Zucker fatty rat model (216). This mutation occurs at the leptin receptor gene, resulting in decreased affinity of leptin for its receptor and, consequently, causing hyperphagia and obesity (217). These fatty rats, although insulin resistant, did not become diabetic, but later inbreeding using obese and diabetic Zucker rats resulted in the Zucker diabetic fatty rat model (218). Both these models develop mild hypertension and cardiac alterations such as LV hypertrophy and early diastolic and moderated systolic dysfunction (17,219–222), with preserved LVEF (223). There is also cardiomyocyte hypertrophy, vascular rarefaction, and fibrosis (220,222–224). However, to our knowledge, lung congestion or other signs of HFpEF have not been described.

Zucker fatty and spontaneously hypertensive HF rat. The diabetic Zucker fatty spontaneously hypertensive heart failure F1 hybrid (ZSF1) model was developed by crossing rat strains with 2 different leptin receptor mutations (fa and fa^{cp}), the lean female Zucker diabetic fatty rat (+/fa) and the lean male spontaneously hypertensive HF rat (+/fa^{cp}), derived from the obese SHR carrying the corpulent fa^{cp} gene (225–227). ZSF1 rats develop HFpEF between 10 and 20 weeks of age. The obese animals die at an early age (~12 months) with symptoms of end-stage renal failure, accompanied by marked cardiac hypertrophy (227). There is hypertension, concentric LV remodeling, progressive diastolic dysfunction, preserved LVEF, lung congestion, and increased arterial stiffness, all followed by insulin resistance, glycosuria, and proteinuria (228–230). Additional studies showed impaired exercise tolerance in ZSF1 rats, reinforcing the potential usefulness of this model to understand HFpEF pathophysiology and for therapeutic insights (231). At the cellular level, there is cardiomyocyte hypertrophy (228). Fibrosis develops later and seems to be induced by a hyperproliferative vascular reaction, which leads to replacement fibrosis of functional vascular structures (229). Interestingly, ZSF1 rats show increased myocardial stiffness, largely due to increased cardiomyocyte stiffness resulting from N2B titin isoform hypophosphorylation (228). This is similar to human HFpEF in which both increased cardiac expression (232) and hypophosphorylation (233) of the stiff N2B titin isoform are described. The use of SU5416 (a vascular endothelial growth factor receptor blocker) in ZSF1 rats induces a “2-hit” model of pulmonary hypertension plus metabolically impairment in HFpEF. There is increased right ventricular systolic pressure, elevated pulmonary vascular resistance, and pulmonary vascular proliferative remodeling (234). Notably, the ZSF1 represents a good model of HFpEF, but in addition to the obesity/metabolic phenotype, it presents other comorbidities such as hypertension or chronic kidney disease, which may be argued to

mimic the HFpEF patient in many scenarios. However, if the aim is to discern the contributions of obesity/metabolic phenotype per se, then these additional comorbidities would confound the findings. It might be a good model to study combinations of comorbidities in advanced studies.

ATRIAL FIBRILLATION. Atrial fibrillation, the most common arrhythmia encountered in clinical practice, is highly associated with an increased risk of HF, stroke, and overall mortality (235). Epidemiological studies show a close relationship between HFpEF and atrial fibrillation, including shared risk factors, such as older age and hypertension (236). Diagnosis of HFpEF in the setting of atrial fibrillation is challenging because of overlapping symptoms, such as dyspnea, fatigue, and impaired exercise tolerance, and comparable echocardiograph parameters, which may include diastolic dysfunction (237) and enlarged left atrial size. Additionally, it is often unclear which is the inciting event, HFpEF or atrial fibrillation, and which of these 2 events should be considered the cause and which the effect.

Historically, cardiac arrhythmias were studied in large mammals such as the goat, pig, or dog, because their hearts were more akin to the human heart than rabbits or rodents (25). Early studies in dogs (238,239) showed that the severity of cardiac dysfunction caused by atrial pacing directly correlates with the rate of pacing. This induces an end-stage dilated cardiomyopathy and an HFrEF phenotype, with a significant decrease in both systolic and diastolic function. There was no increase in LV mass nor collagen content (21).

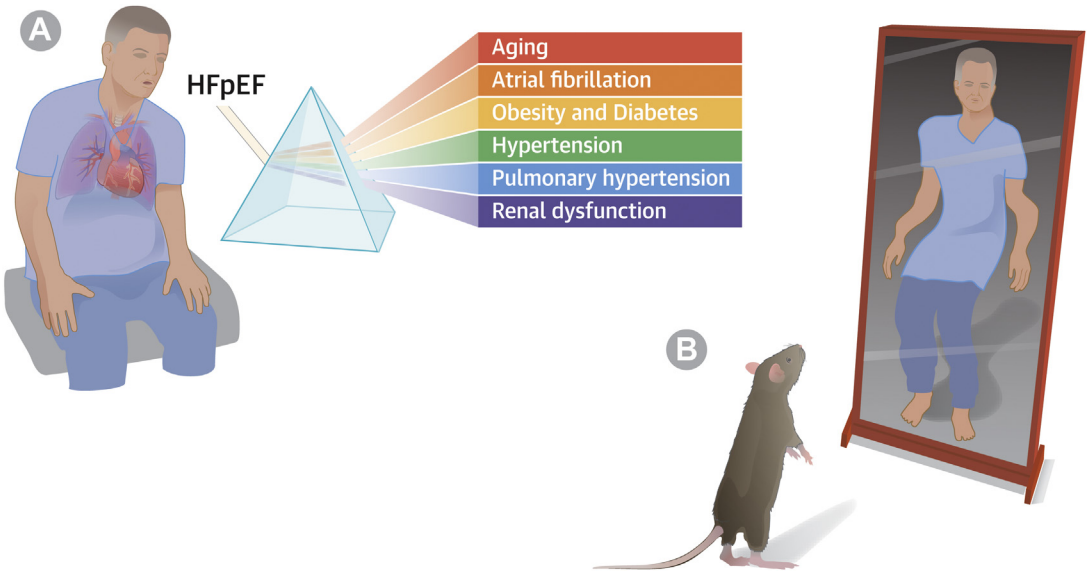
Although it was believed that arrhythmias did not occur in mice due to their lack of critical cardiac mass (240), this has been shown to be incorrect (241). Studying atrial fibrillation is difficult compared with other HFpEF comorbidities. Numerous murine models are reported to harbor atrial arrhythmias, and in vivo electrophysiological techniques (242–244) are well established. Transgenic mouse models showing the development of atrial fibrillation, secondary to alterations in major regulatory pathways of cell functions, have been broadly reviewed (245). In these, atrial fibrillation is often associated with alterations in a substrate for atrial conduction, electrophysiological abnormalities that accelerate atrial repolarization, or triggered activity and focal discharges (246). However, to our knowledge, although some of these models show dilated cardiomyopathy or hypertrophic cardiomyopathy, information regarding HF status is lacking, and none have examined the presence of HFpEF in the setting of atrial fibrillation.

PULMONARY HYPERTENSION. Pulmonary hypertension is a known complication of any disease that elevates LV filling pressure (247), including LV systolic (248) and diastolic dysfunction (249). For reasons that are yet uncertain, a subset of patients with HFpEF go on to develop pulmonary hypertension (250). The clinical pulmonary hypertension phenotype associated with HFpEF varies and may be seen in acute or chronic HF. However, when pulmonary hypertension is evident in chronic HF, its clinical significance is tightly linked to increased disease severity and adverse outcomes (251).

Pulmonary hypertension in animal models may not fully represent the clinical observations. It has been argued that the severity of pulmonary hypertension in vivo poorly mimics the observed human phenotype (252). Preclinical models of pulmonary hypertension may reflect milder forms of human pulmonary hypertension, a stage that is often missed at the time of diagnosis (253), and thus do not represent the later, more severe, disease (252). There are strain issues with murine models which recapitulate the human phenotype of metabolic syndrome and pulmonary hypertension-associated HFpEF. The latter is only seen in select mouse strains (284). Nevertheless, animal models have undergone major development and improvement over the years, and pulmonary hypertension models are more common nowadays, although, as noted, the pulmonary changes observed are not completely comparable between rodents and humans (254).

Hypoxia-induced pulmonary hypertensive rat and mouse. Chronic hypoxia is observed in chronic obstructive pulmonary disease, interstitial lung disease, sleep apnea, and exposure to high altitudes in humans and is thought to play a crucial role in the development of pulmonary hypertension and severe HF (252,255). Similarly, in the research laboratory, chronic hypoxia also leads to pulmonary hypertension (255). Typically, it is induced by placing the rodent in a hypobaric chamber (either 10% fraction of inspired oxygen or hypobaric pressure of 380 mm Hg, which is equivalent to one-half that at sea level) for 3 to 4 weeks (256–259). This model is useful because it is predictable and reproducible within a selected animal strain. There is also variability in the responses to chronic hypoxia between species (260). Hypoxia in rats produces characteristic morphological changes in the pulmonary vasculature, including an extension of smooth muscle into more peripheral pulmonary arteries and reduction in their number (261). Nevertheless, exposure of mice to chronic hypoxia conditions, although causing an elevation in

CENTRAL ILLUSTRATION HFpEF



Valero-Muñoz, M. et al. J Am Coll Cardiol Basic Trans Science. 2017;2(6):770–89.

(A) Modeling heart failure with preserved ejection fraction (HFpEF) in the lab with major comorbidities and conditions highly associated with human HFpEF. **(B)** However, murine models touted as HFpEF models are often lost in translation when applied to the clinical situation.

pulmonary artery pressure, is associated with less vascular remodeling than in rats (262-264). Although the mechanisms of hypoxia-induced vascular remodeling are partially understood, the complex obliterate lesions found in human patients with severe primary pulmonary hypertension do not develop in this rodent model (265). Instead of these obliterate lesions in the pulmonary vasculature of humans with left-sided heart disease, HF and pulmonary hypertension (266), there is evidence of alveolar septa thickening, cellular proliferation, and collagen deposition (234). It has been described that chronic hypoxia causes right ventricle (258,259) and LV (267,268) hypertrophy in rats/mice, but to our knowledge there are no in vivo studies of HFpEF induced by chronic hypoxia, although this might be an applicable model of pulmonary hypertension-associated HFpEF (62,260).

RENAL DYSFUNCTION PHENOTYPE. HFpEF and renal dysfunction are mutually promoting (6). Abnormalities in the kidney's ability to maintain sodium and fluid balance may precede the development of HFpEF. Evidences suggest a putative role for the kidney in HFpEF as a potential starting point in patients vulnerable to the deleterious effect of excessive salt and water retention, such as in hypertension (269). Conversely, the detrimental impact of HFpEF on renal function might be explained by the effect of increased filling pressures, typical for HFpEF, entailing both a relative decreased cardiac output and increased renal congestion. This, together with an impaired ventricular-vascular coupling, can lead to reduced renal blood flow and renal dysfunction (270,271).

Subtotal nephrectomy rat and mouse. Often termed 5/6 nephrectomy, it is the most established model of progressive renal failure with loss of renal mass. Although commonly performed in the rat, a similar mouse model exists but appears to be less reliable and more strain dependent (272,273). The model is based on the reduction of renal mass with a uninephrectomy and resection of the poles of the contralateral kidney (approximately 50%) 1 to 2 weeks later (274). Rather than mimicking a renal disease per se, subtotal nephrectomy parallels the consequences of reducing functional nephron number. In this model, LV hypertrophy is a consistent feature (275) and resembles that seen in early chronic kidney disease in humans (276). The presence of LV dilatation is variable (273), and cardiac systolic function is generally maintained, with preserved LVEF. Early diastolic dysfunction is seen with a commensurate increase in cardiomyocyte cross-sectional area (277-279). Other documented features of this model include increased

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myocardial artery wall thickness, decreased capillary density (280), interstitial fibrosis (277,279), fluid congestion (281), and exercise intolerance (282). However, to our knowledge, it has not been used as a model of HFpEF associated renal disease and its significance in modeling HFpEF is unknown.

MURINE MODELS: A FUNHOUSE MIRROR?

Relevant preclinical models for HFpEF will provide mechanistic information to understand the contribution of comorbidities that are highly associated with this disease. Additionally, these models will provide insights into pathways relevant to disease development, progression, and potential implications in therapeutics. As expected, an animal model cannot completely mimic the human disease, and limitations regarding their applicability are obvious, partly because human HFpEF is so heterogeneous and encompasses a large spectrum of symptoms, signs, and disease presentation (62). It is therefore important for the translational researcher to carefully select which comorbidity or combination thereof, leading to this heterogeneous HFpEF syndrome, should be included in the experimental design. A general limitation of most HFpEF murine models is the sudden onset of the HF due to a surgical or drug intervention, whereas human HFpEF is generally thought to develop progressively over months to years (23). Moreover, substantial differences exist between rodents and human cardiac features, such as its small size and higher heart rates, which sometimes limit diastolic function measurements, especially when noninvasive techniques, such as echocardiography, are used (103,283). It should also, be emphasized that many murine models of purported HFpEF progress to HFrEF within a variable amount of time, suggesting that in these models, HFpEF is a merely a temporary step to the development to HFrEF, which is rare in human HFpEF (100,103).

The use of murine models is an extremely valuable tool in understanding the pathophysiology of HFpEF. These models are essential for understanding the molecular alterations underlying the development of the disease, as they allow the identification of molecular pathways that may be causative or contribute to the pathophysiology and progression of HFpEF (23,103). However, it is essential for these models to be validated on the basis of reliability and reproducibility in multiple experimental settings, including time-course analysis. Attention should be also paid to the background strain of the model, as different stimuli may elicit a variable response depending on the strain (284). It has been suggested that similar to human HFpEF, the use of outbred murine colonies could contribute to a better experimental setting because, in contrast, the use of inbred strains represents limited genetic diversity and might not reflect the responses generated in a diverse human population (285).

Importantly, HFpEF models must be carefully characterized to ensure they have features consistent with human HFpEF (**Figure 1**). It is likely that some murine models in the present review do have HFpEF, but have not been described. Conversely, others touted as HFpEF models do not present the real HF phenotype. Similarly, in the preclinical literature diastolic dysfunction has often been used incorrectly as interchangeable with the term diastolic heart failure and thus thought to represent HFpEF.

Lastly, although recent technological advances in echocardiography, imaging, and micromanometer conductance catheters have greatly improved the assessment of cardiac function in rodents (23), they cannot be the only method for HFpEF diagnosis, as they may not reflect a real HF syndrome. For this reason, the presence of HF in vivo should be more accurately detected by determining exercise intolerance (17). The recommended method for post-mortem identification of HFpEF is determining pulmonary congestion, which could be assessed by examining either the ratio of lung weight to tibia length or the wet-to-dry lung ratio (17).

As with all fishing expeditions, we attempt to gather facts about HFpEF using relevant preclinical models that accurately recapitulate the complexities of human HFpEF disease and define specific phenotypes to discover novel targets, ultimately for the development of effective therapeutics (**Central Illustration**).

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KEY WORDS comorbidities, HFpEF, murine model