

STATE-OF-THE-ART REVIEW

What Is Cardiometabolic HFpEF and How Can We Study it Preclinically?



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HIGHLIGHTS

- **Cardiometabolic heart failure with preserved ejection fraction (HFpEF) has emerged as a distinct and dominant endotype within the broader spectrum of HFpEF.**
- **Cardiometabolic HFpEF is characterized by unique clinical characteristics and molecular pathophysiology.**
- **Development and rigorous characterization of clinically relevant animal models that mirror human cardiometabolic HFpEF are essential for advancing mechanistic understanding and therapeutic discovery.**
- **Future research should emphasize the use of appropriate animal models of cardiometabolic HFpEF.**

SUMMARY

Cardiometabolic heart failure with preserved ejection fraction (HFpEF) has emerged as a distinct and dominant endotype of HFpEF. Driven by prevalent comorbidities, its incidence and prevalence are projected to continue to rise amid the current global pandemic of obesity and metabolic disease. Recognizing the characteristic clinical and molecular features of cardiometabolic HFpEF is paramount for developing an efficacious therapeutic arsenal and improving clinical outcomes, challenges in which success to date has been modest. Studying relevant and clinically informative animal models of cardiometabolic HFpEF can afford valuable insights into the molecular underpinnings of this syndrome, allowing the possibility of novel advances with clinical relevance. Here, we outline the clinical and molecular features that define cardiometabolic HFpEF as a distinct endotype. We also discuss the bona fide animal models of cardiometabolic HFpEF currently available, as well as methods for developing new models. (JACC Basic Transl Sci. 2025;10:101295) © 2025 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/>).

Hear failure (HF) is a rapidly expanding public health concern on a global scale; its prevalence in the United States alone is estimated to reach >8 million cases (1 in 33 adults) by 2030 with a total cost of \$70 billion.^{1,2} Heart failure with preserved ejection fraction (HFpEF) constitutes more than half of HF cases and its prevalence is increasing relative to heart failure with reduced

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Manuscript received July 31, 2024; revised manuscript received October 3, 2024, accepted April 14, 2025.

**ABBREVIATIONS
AND ACRONYMS**

| | |
|--------------------------|--|
| AB | = surgical aortic banding |
| Ang II | = angiotensin II |
| db/db | = leptin receptor-deficient mice |
| EAT | = epicardial adipose tissue |
| EF | = ejection fraction |
| eNOS | = endothelial nitric oxide synthase |
| GLS | = global longitudinal strain |
| HFD | = high-fat diet |
| HFpEF | = heart failure with preserved ejection fraction |
| HFrEF | = heart failure with reduced ejection fraction |
| iNOS | = inducible nitric oxide synthase |
| L-NAME | = N ^ω -nitro-L-arginine methyl ester |
| ob/ob | = leptin-deficient mice |
| TTE | = transthoracic echocardiography |
| VO_{2max} | = Maximal oxygen uptake |
| WD | = Western diet |

ejection fraction (HF_rEF).^{3,4} Mounting evidence points to HFpEF as a comorbidity-driven multisystemic clinical syndrome, and there is a growing consensus that these comorbidities are not merely associated with HFpEF but actively drive disease pathophysiology. Furthermore, the HFpEF population is a heterogeneous group of patients that manifests differences in comorbidity burden, disease severity, cardiac structure, circulating biomarkers, and prognosis.⁵⁻⁸ These facts alone highlight challenges associated with developing and optimizing preclinical models of HFpEF which are critical to the development of clinically meaningful advances.

Metabolic comorbidities including obesity, diabetes, and hypertension are highly prevalent among patients with HFpEF and compelling evidence argues that cardiometabolic HFpEF merits recognition as a distinct endotype of HFpEF.^{3,9,10} When compared with other pathophysiological subtypes, cardiometabolic HFpEF is characterized by more severe symptom burden and reduced quality of life.^{11,12} It is now recognized that metabolic comorbidities drive

disease pathophysiology by inducing a host of multisystemic and myocardial alterations. As the prevalence of obesity, type 2 diabetes, and metabolic syndrome have risen dramatically over the last several decades, reaching “syndemic” proportions,¹³ cardiometabolic HFpEF is expected to become an increasingly prevalent syndrome.¹⁴

Given the substantial morbidity and mortality associated with cardiometabolic HFpEF and currently limited therapeutic options, it is crucial to decipher mechanisms contributing to disease pathogenesis. This has heightened the need for reliable, bona fide preclinical models that faithfully replicate the clinical features of this syndrome. In this review article, we explore the clinical characteristics of cardiometabolic HFpEF and the molecular mechanisms underlying its pathophysiology. We examine existing preclinical models and discuss the methodologies used to develop and characterize new models of cardiometabolic HFpEF. Our goal is to provide a comprehensive understanding of this distinct endotype, highlighting the current state of research and ways to develop and optimize novel models to expand our understanding of this grievous syndrome.

HFpEF: A CLINICAL PERSPECTIVE

HISTORICAL BACKGROUND. During the 20th century, an increasingly granular understanding of cardiovascular physiology began to emerge, highlighted by key advances such as those of Starling in 1918, the advent of cardiac catheterization in the 1940s, and Sarnoff’s work on contractility in the 1950s. These milestones laid the groundwork for elucidating the hemodynamic abnormalities in HF. As early as 1937, Fishberg¹⁵ identified 2 types of HF: hypo-diastolic and hypo-systolic failure. Despite this early observation, for much of the 20th century HF was predominantly viewed through the lens of overt systolic dysfunction. Cardiac contractility and ventricular systolic function became the centerpiece of HF research, with ejection fraction (EF) emerging as the clinical gold standard for assessing systolic function.

In the 1980s, it was noted that some patients exhibited similar clinical HF symptoms without overt systolic dysfunction setting the stage for what would be termed “diastolic heart failure.”¹⁶⁻¹⁸ Reports by Topol et al¹⁹ and Given et al²⁰ detailed the presence of a subgroup of patients manifesting HF symptoms with concomitant severe hypertension, concentric left ventricular (LV) remodeling, and a preserved EF in the absence of ischemic heart disease. This syndrome was termed “congestive heart failure and preserved ventricular systolic function” by the latter group. It was noted that these patients manifested diastolic dysfunction as evidenced by a prolonged early diastolic filling period and a reduced peak diastolic dimension.¹⁹ Furthermore, it was suggested that the concentrically hypertrophied LVs displayed increased diastolic stiffness resulting in elevated diastolic pressures.²⁰ Recognition that diastolic dysfunction was the driving hemodynamic abnormality for HF began to grow in the latter part of the 1980s, as the term diastolic heart failure began to appear in the literature. Nevertheless, doubts persisted until the early 2000s regarding the hemodynamic abnormalities driving disease pathogenesis. During this period, Zile et al²¹ showed that diastolic dysfunction was the pathophysiological cause of elevated diastolic LV pressures and HF. The last decade has seen our view of HFpEF as a syndrome caused solely by abnormalities in diastolic function shift to an understanding that HFpEF is a comorbidity-driven, systemic, clinical syndrome with multiple subtypes.

Today, HFpEF is defined as a clinical diagnosis of HF with an EF \geq 50% in the absence of an attributable

cause.²² HFpEF diagnostic scoring systems, such as H₂FPEF and HFA-PEFF, can aid the diagnosis of HFpEF, which ultimately requires either direct or inferred proof of increased cardiac filling pressures at rest or with exercise.^{22,23} It is important to recognize that cardiovascular and noncardiovascular mimics of HFpEF can present with symptoms of HF and an EF \geq 50% but are distinct pathologies.^{22,23} These syndromes with pathophysiology distinct from HFpEF should not be conflated with HFpEF. In fact, we submit that the inadvertent inclusion of these and other syndromes in HFpEF clinical trials—admixing multiple different disease pathologies—has likely contributed to the numerous failures in this space. Cardiac mimics of HFpEF include infiltrative diseases such as amyloidosis and sarcoidosis, hypertrophic cardiomyopathy, and constrictive pericarditis. Noncardiac mimics include chronic kidney disease (CKD), nephrotic syndrome, liver failure, and non-type II pulmonary hypertension.^{22,23} Of note, some features of the above syndromes may exist as comorbidities within clinical HFpEF. For example, a significant proportion of HFpEF patients also suffer from CKD. This overlapping pattern makes the clinical diagnosis of HFpEF challenging.

CLASSIFICATION OF THE HFpEF SYNDROME. Considering our limited success in treating HFpEF, two important points merit emphasis. First, despite exclusion of attributable causes and mimics in establishing the diagnosis of HFpEF, we are left with a heterogenous population of patients who, while displaying similar clinical manifestations, can harbor widely differing pathophysiological mechanisms. Second, HFpEF is a comorbidity-driven clinical syndrome impacting multiple organ systems. Clearly, diastolic dysfunction is a prominent feature of HFpEF; however, unquestionably, HFpEF is far more than just diastolic dysfunction. Diastolic dysfunction can emerge from a number of different pathophysiological triggers as evidenced by the multiple preclinical models of HFpEF involving a wide range of cardiovascular interventions.²⁴ It may be posited that HFpEF is a blanket diagnosis covering a heterogenous population of patients presenting with clinical HF and a preserved EF but harboring fundamentally different disease pathogenesis. This, then, begs the question whether there is a better way to subclassify the HFpEF population.

Cardiometabolic disorders are a cluster of comorbidities that include obesity, dyslipidemia, hypertension, and diabetes that are strongly associated with the occurrence of cardiovascular diseases.²⁵ Although cardiometabolic disorders are strongly

associated with HF incidence and adverse outcomes, an international cohort study involving more than 20,000 participants reported that cardiometabolic traits including obesity, insulin resistance, and waist circumference, a surrogate for visceral adiposity, are more strongly associated with a risk of developing HFpEF than HFrEF.²⁶⁻²⁹ A large majority of HFpEF patients are overweight or obese and hypertensive with cardiometabolic comorbidities.³⁰⁻³³ In these patients, cardiometabolic comorbidity-driven pathophysiological mechanisms drive disease. These findings are mirrored in various preclinical models of cardiometabolic HFpEF which report consistently that inducing obesity and hemodynamic overload is sufficient to elicit HFpEF. Weight loss via caloric restriction and exercise training in HFpEF patients reduced cardiac remodeling, symptom severity, and NYHA functional class while improving exercise capacity.³⁴ Weight loss is a powerful strategy for reducing blood pressure, insulin resistance, and adipose tissue inflammation, and recent evidence points to symptomatic benefit deriving from glucagon-like peptide-1 agonists, a pharmacological strategy that triggers robust weight loss.^{35,36}

Considerable evidence derived from baseline characterization of the HFpEF population now supports the notion that cardiometabolic HFpEF is the predominant endotype of HFpEF.³⁰ The distinct nature of this endotype can be established based on its marked clinical severity, prominent maladaptive structural changes, and unique molecular signature. Unfortunately, under-enrollment of patients with cardiometabolic HFpEF has continued in major HFpEF trials.³⁷ Treating cardiometabolic HFpEF as a unique subgroup, distinct from noncardiometabolic HFpEF, in preclinical studies and clinical trials may be a steppingstone to better elucidating the underlying pathophysiology and discovering relevant therapeutic targets with clinical relevance.

CLINICAL CHARACTERISTICS OF CARDIOMETABOLIC HFpEF.

Patients with cardiometabolic HFpEF are marked by greater symptomatic burden with worse peripheral edema and orthopnea, worse NYHA functional classification, and reduced quality of life when compared to patients with noncardiometabolic HFpEF.^{11,12} The disease burden seen in these patients is reflected in the more severe features observed with various clinical testing modalities. A handful of comparative multimodality studies have been performed to delineate unique characteristics of cardiometabolic HFpEF.^{11,38,39} Perhaps the first and most comprehensive of these by Obokata et al³⁸ involved a phenotyping study comparing non-obese (body mass

index [BMI] <30 kg/m²) and class II obese (BMI ≥35 kg/m²) HFpEF patients, uncovering distinct differences between the 2 groups thereby making a strong case for the existence of a unique cardiometabolic endotype of HFpEF. Aggregating these various studies has yielded a consistent pattern of more severe hemodynamic abnormalities, diminished functional capacity, and a unique biomarker profile characterized by lower N-terminal pro-B-type natriuretic peptide and increased inflammatory markers when compared to noncardiometabolic HFpEF.

Cardiopulmonary studies, both invasive and noninvasive, have identified differences in ventricular structure, systolic and diastolic performance, and hemodynamics between cardiometabolic and noncardiometabolic HFpEF. Striking is the consistently more severe right ventricular (RV) and LV maladaptive remodeling observed by echocardiography in cardiometabolic as opposed to noncardiometabolic HFpEF.^{11,38,39} Whereas LV diastolic parameters of E/e' and E/A, and left ventricular ejection fraction (LVEF) manifested no difference between cardiometabolic and noncardiometabolic endotypes, more sensitive measures of systolic and diastolic dysfunction detected by global longitudinal strain (GLS) and early diastolic global longitudinal strain rate, respectively, are exacerbated in cardiometabolic HFpEF.^{11,38,39} RV systolic function measured by fractional area change is lower in cardiometabolic HFpEF.³⁸ Resting invasive hemodynamic studies revealed elevated right atrial pressures and pulmonary capillary wedge pressure in cardiometabolic HFpEF whereas other parameters were similar between groups.³⁸ These changes were observed in the setting of increased plasma volume in cardiometabolic HFpEF.³⁸ External compressive forces exerted by the pericardium and RV contribute substantially to intracavitary LV pressure. Increased epicardial adiposity and ventricular hypertrophy result in pericardial restraint and more prominent ventricular interaction in cardiometabolic HFpEF patients.³⁸

Functional capacity impairment and exercise intolerance, each characteristic of HFpEF, are more pronounced in cardiometabolic HFpEF. These patients display reduced peak oxygen consumption (VO₂) and diminished ability to translate metabolic work into ergometric work when compared with noncardiometabolic HFpEF.^{38,40} Exercise elicits higher left and right heart filling pressures in cardiometabolic HFpEF compared with noncardiometabolic HFpEF whereas exercise-induced pulmonary hypertension and exercise blood pressure are higher. Chronotropic incompetence is also more

pronounced in this patient group.³⁸ The pathophysiology of exercise intolerance in HFpEF is multifactorial with skeletal muscle changes also contributing. The pattern of adipose tissue distribution, specifically intra-abdominal and intramuscular adipose tissue depots, may be an important element in exercise intolerance in cardiometabolic HFpEF.^{40,41}

Although atrial fibrillation is highly prevalent in all types of HFpEF, it is less common in cardiometabolic versus noncardiometabolic HFpEF.^{11,38,39} Absolute left atrial volume is similar between cardiometabolic and noncardiometabolic HFpEF; however, when normalized to body surface area, left atrial volume index is lower in cardiometabolic HFpEF patients.¹¹

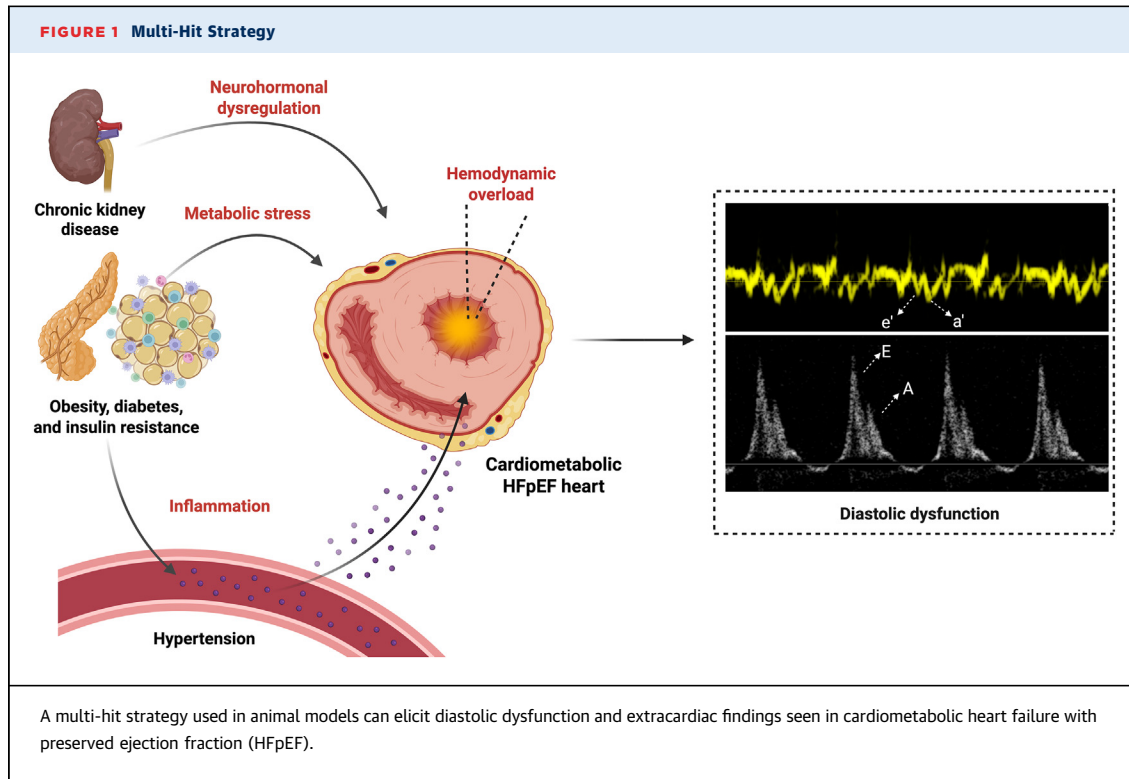
The biomarker profile of cardiometabolic HFpEF patients similarly manifests unique features as evidenced by lower N-terminal pro-B-type natriuretic peptide despite more severe symptoms, increased inflammatory markers, and higher uric acid levels.^{11,12,38} Endotrophin is also emerging as a unique biomarker for cardiometabolic HFpEF being associated with worse outcomes.⁴²

Although obesity, as measured by BMI, is an independent risk factor for the development of HF, increasing BMI is associated with reduced mortality in HF patients, a phenomenon termed the “obesity-survival paradox”.⁴³ A recent study has questioned this paradox in HFpEF; using different anthropometric measures of obesity, this paradox was eliminated.⁴⁴ No such study has been performed in HFpEF.

PRECLINICAL MODELS OF CARDIOMETABOLIC HFpEF

It is important to emphasize that developing and analyzing preclinical models that recapitulate the clinical realities of cardiometabolic HFpEF is vital to the discovery of pathophysiological mechanisms specific to the syndrome. While developing such models, it is paramount to bear in mind the comorbidity-driven nature of cardiometabolic HFpEF and the clinically encountered features of the syndrome such as the extracardiac manifestations that define any type of HF, namely, edema and exercise intolerance. Here, we summarize important features that we deem essential to model clinical cardiometabolic HFpEF in vivo, and the phenotyping tools available to assess and validate these features.

Preclinical models of cardiometabolic HFpEF have largely relied on a “multi-hit” strategy using different interventions, including genetic manipulation, surgery, and diet modification to induce metabolic stress, and hemodynamic overload in small and large



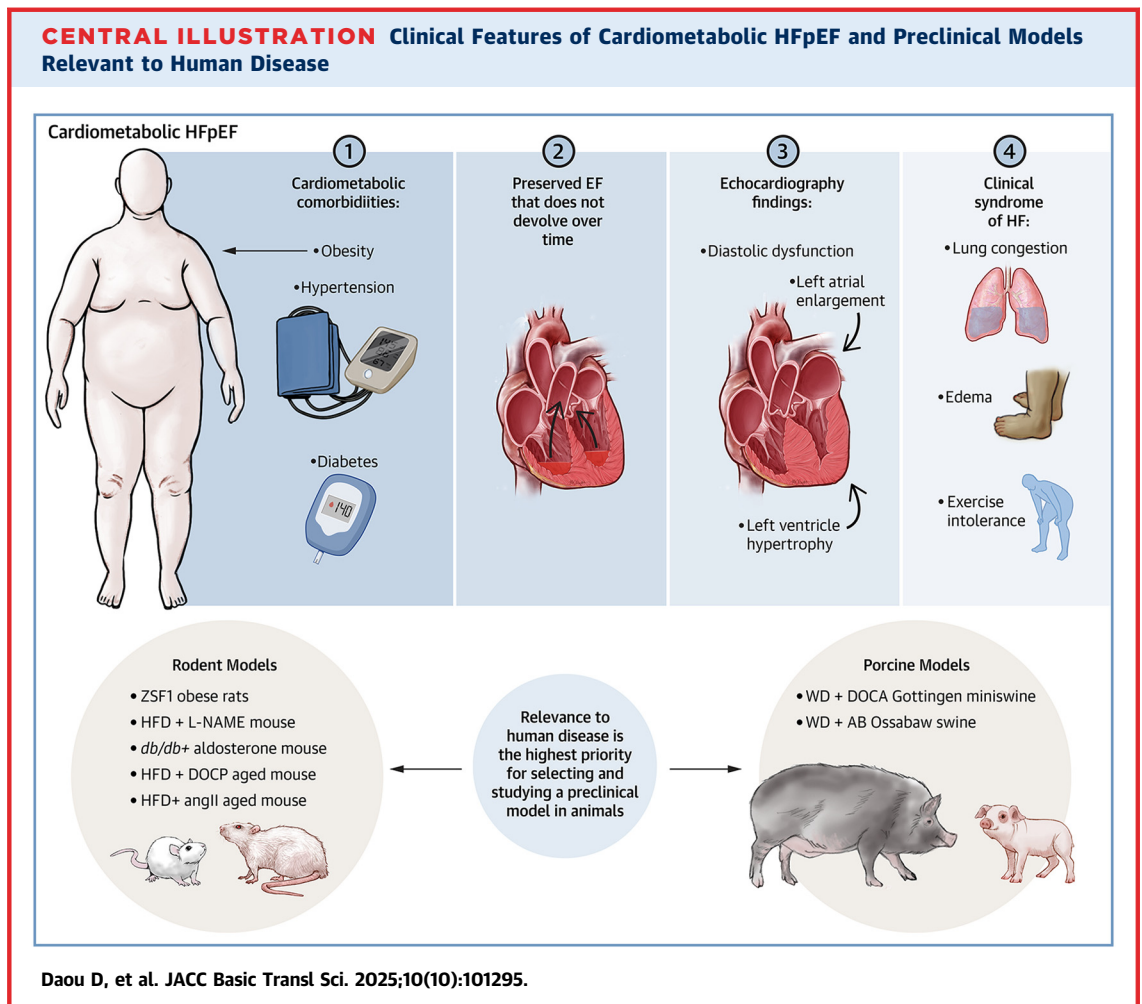
animal species (Figure 1). Over time, a trend has emerged to move away from single-hit to multi-hit models as the latter more accurately recapitulate human cardiometabolic HFpEF (Table 1).

Cardiometabolic HFpEF is driven by comorbidities and lifestyle. Although genetic predispositions undoubtedly play a role in developing disease, cardiometabolic HFpEF is not a single-gene, Mendelian

TABLE 1 Assessment of Animal Models of HFpEF

| | Obesity | HTN | T2DM | Diastolic dysfunction | LVH | Left Atrial Enlargement | Pulmonary Edema | Exercise Intolerance | Score |
|----------------------------------|---------|-----|------|-----------------------|-----|-------------------------|-----------------|----------------------|-------|
| Cardiometabolic HFpEF | | | | | | | | | |
| ZSF1 obese rat | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | 8 |
| HFD+ L-NAME mouse | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | 8 |
| db/db + aldosterone mouse | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | 8 |
| HFD + DOCP aged mouse | ✓ | ✓ | ✓ | ✓ | ✓ | N/A | ✓ | ✓ | 7 |
| HFD + Ang II aged mouse | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | N/A | 7 |
| WD + DOCA Göttingen miniswine | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | N/A | 7 |
| WD + AB Ossabaw swine | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | N/A | 7 |
| Noncardiometabolic HFpEF | | | | | | | | | |
| db/db | ✓ | × | ✓ | ✓ | ✓ | ✓ | × | ✓ | 6 |
| Spontaneously hypertensive rats | × | ✓ | × | ✓ | ✓ | ✓ | ✓ | ✓ | 6 |
| Dahl-salt sensitive rats | × | ✓ | × | ✓ | ✓ | ✓ | ✓ | ✓ | 6 |
| ob/ob | ✓ | × | ✓ | ✓ | ✓ | × | × | ✓ | 5 |
| Aldosterone uninephrectomy mouse | × | ✓ | × | ✓ | ✓ | N/A | ✓ | ✓ | 5 |
| Ang II infusion mouse | × | ✓ | × | ✓ | ✓ | N/A | ✓ | ✓ | 5 |
| SAMP 8 mouse | × | ✓ | × | ✓ | ✓ | ✓ | × | ✓ | 5 |
| Aged mice (24-30 mo) | × | × | × | ✓ | ✓ | N/A | ✓ | ✓ | 4 |
| DOCA salt-sensitive model | × | ✓ | × | ✓ | ✓ | N/A | × | × | 3 |

Ang II = angiotensin II; db/db = leptin receptor deficient mice; DOCA = deoxycorticosterone acetate; HFD = high-fat diet; HFpEF = heart failure with preserved ejection fraction; HTN = hypertension; L-NAME = N^o-nitro-L-arginine methyl ester; LVH = left ventricular hypertrophy; ob/ob = leptin deficient mice; N/A = not applicable; SAMP = senescence-accelerated mouse-prone; T2DM = type 2 diabetes mellitus; ZSF1 = Zucker fatty/spontaneously hypertensive heart failure F1 hybrid.



disorder such as certain hereditary cardiomyopathies. As such, we argue that preclinical models of HFpEF that derive from genetic alterations typically of unknown molecular basis are suboptimal in the modeling of the syndrome. In our work, we have chosen to avoid these models (eg, leptin-deficient (*ob/ob*) mice, leptin receptor-deficient (*db/db*) mice, spontaneously hypertensive rats, Zucker fatty/spontaneously hypertensive heart failure F1 hybrid (ZSF-1) obese rats, etc).

A preclinical model of cardiometabolic HFpEF faithful to the clinical realities of the syndrome should incorporate the following features (**Central Illustration**): 1) presence of cardiometabolic comorbidities, including obesity, metabolic syndrome, possibly diabetes, and hypertension; 2) diastolic dysfunction with a preserved EF that does not devolve into reduced EF over time (as the vast majority of HFpEF patients do not transition to HFrEF)⁴⁵; 3) echocardiographic findings consistent

with human HFpEF including LV hypertrophy and left atrial enlargement; and 4) a clinical syndrome of HF, which is marked by extracardiac manifestations that include edema and exercise intolerance.

PRECLINICAL MODEL DEVELOPMENT. Animal species. It is important for any investigator aiming to develop such a model to select suitable animal species and treatment conditions. Existing HFpEF models can be broadly categorized as small or large animal models. Small animal models are typically rodent species. They are cost-effective, easy to manipulate genetically, and have shorter gestation times allowing for high throughput studies. Large animal models of HFpEF are typically porcine species. Whereas mammals share similar cardiovascular physiology, human and porcine species have especially similar cardiovascular anatomy and physiology, making them attractive models in cardiovascular research.⁴⁶ They

are, however, more expensive, rendering financial feasibility a challenge. Of all species, nonhuman primates are most similar to humans; however, strict regulations due to ethical considerations are a major barrier to their use in research. Other animal species such as canine species, rabbits, hamsters, and guinea pigs have been used in cardiovascular research. Strain differences within the same species and within-strain differences are also important to consider.

Cardiometabolic comorbidity phenotyping. Cardiometabolic comorbidities including obesity, insulin resistance, and hypertension are critical components of the cardiometabolic HFpEF syndrome. It is important to assess the presence of these comorbidities when creating an animal model of cardiometabolic HFpEF. **Obesity.** Tracking weight over time is important to ensure adequate weight gain. Normal body weight parameters and weight gain are species- and strain-dependent. Other measures used to track obesity may include the Lee index, BMI, and fat pad mass.⁴⁷ Dual-energy x-ray absorptiometry and time-domain nuclear magnetic resonance can be used to measure body mass composition.⁴⁸

Insulin resistance. Metabolic tolerance tests are important phenotyping tools used to measure insulin resistance and glucose intolerance. These assays are easy to perform and are reproducible. In glucose tolerance tests, a period of fasting is followed by a glucose bolus administered via intraperitoneal, intravenous, or oral routes, and serial measurements of blood glucose are made at different time points.⁴⁹⁻⁵¹ Insulin tolerance tests are also commonly used and begin with a period of fasting followed by intraperitoneal administration of an insulin bolus and serial measurements of blood glucose.⁴⁹⁻⁵¹ The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and the Qualitative Insulin Sensitivity Check Index (QUICKI) can also be used in murine species as an indirect measure of insulin resistance.^{52,53}

More invasive tests can be used to evaluate insulin resistance and glucose tolerance including hyperglycemic clamps and hyper-insulinemic-euglycemic clamps that rely on surgical catheterization for blood sampling. Route of glucose bolus administration, age, strain, and sex of animals are important factors to be considered as they can alter glucose metabolism.⁵¹ Even animals of the same strain obtained from different suppliers may manifest differences in insulin secretion.^{54,55} Stress and anesthetic agents can also alter glucose homeostasis.^{51,56,57} Isoflurane, a frequently used anesthetic in murine echocardiography, can cause suppression of insulin secretion.⁵⁸ Minimizing animal stress and avoiding the use of anesthesia during or before performing

metabolic assays is important. Performing assays at a consistent time of day is another factor to consider as various aspects of glucose metabolism oscillate in a circadian fashion.^{51,59}

Hypertension. Assessment of hypertension can be performed directly via radiotelemetry or indwelling catheters, and indirectly via limb or tail cuff depending on the species under study.⁶⁰ Despite larger variability and potentially lower accuracy, indirect methods are noninvasive, less expensive, more accessible, and allow repeated measurements over time. In murine species, animal handling and restraint can induce significant stress; therefore, handling animals with care, reducing external stimulation, and providing several days of acclimatization may improve reading accuracy with tail-cuff blood pressure measurements.⁶⁰⁻⁶² Warming mice using a heating platform to increase blood flow to the tail and control for ambient room temperature, which is typically below the thermoneutral range for mice, is recommended by machine manufacturers and some studies.^{62,63} Because murine species are nocturnal, diurnal variation in blood pressure is such that measurement during daylight working hours does not accurately reflect mean blood pressure.⁶⁰ Consistently measuring blood pressure at a similar time of day may help control for circadian variations when comparing readings between groups and when tracking blood pressure over time. Anesthetics should be avoided before or during blood pressure measurements.^{60,64} Limb and tail-cuff blood pressure measurements have also been described as reliable methods for porcine species.⁶⁵⁻⁶⁸ Direct measurements are the gold standard for measuring blood pressure and allow for continuous monitoring without the need to restrain animals; however, they are more expensive and invasive.⁶⁰ In animal models using surgical methods to induce pressure overload, measuring systemic blood pressure may not be appropriate.

Cardiac function phenotyping. Systolic function. A preserved LVEF that does not decline with time is a hallmark of HFpEF. Transthoracic echocardiography (TTE), cardiac magnetic resonance imaging (MRI), and invasive hemodynamic testing can be used to assess systolic function.

TTE can be performed to measure LV interventricular septal thickness, left ventricular internal dimensions, and posterior wall thickness in systole and diastole.^{69,70} These measurements are used to calculate fractional shortening and EF. Whereas measures of overt systolic function are preserved, more sensitive measures such as GLS are impaired in HFpEF.⁷¹ B-mode traces from different views can be acquired

using TTE and analyzed using speckle-tracking software to obtain GLS. Cardiac MRI can also be performed to obtain EF, fractional shortening, and GLS. There are no standardized guidelines for animal echocardiography.

Diastolic function. Diastolic dysfunction is a defining feature of HFpEF and can be assessed using various modalities including TTE, MRI, and invasive hemodynamics. All 3 approaches can be performed with small and large animals.

TTE can be performed to obtain multiple parameters of diastolic function including E/A ratio, deceleration time of early filling of mitral inflow, isovolumetric relaxation time, and isovolumetric contraction time. This can be combined with tissue Doppler to obtain an E/e' ratio. These studies are usually performed on anesthetized animals as modest slowing of heart rate is required to extend the diastolic period. These parameters can also be obtained using cardiac MRI. Invasive hemodynamic studies are the gold standard when assessing diastolic function; however, they are less accessible and more invasive. These studies can be performed on murine and porcine species to obtain information on LV volumes, filling pressures, contractile and relaxation forces, and derivative measures such as LV minimum pressure rates (dP/dt_{min}) and isovolumetric relaxation constant (τ).

Extracardiac phenotyping. Dyspnea, edema, and exercise impairment are defining features of all types of HF, and assessment of these features by surrogate measurements is important in any animal model of HFpEF. Absence of these features means the model cannot be regarded as a bona fide HFpEF model.

Pulmonary edema can be assessed by obtaining the ratio of lung weight to tibia length, lung weight to body weight, or wet lung weight to dry lung weight. Microscopic techniques may provide useful qualitative evidence of pulmonary edema.⁷² Pulmonary vascular remodeling and pulmonary hypertension are frequently encountered in HFpEF patients. Right heart catheterization can be used to measure right ventricular systolic pressure which can be used to estimate mean pulmonary artery pressure. Echocardiography to estimate pulmonary hypertension non-invasively can be performed by measuring pulmonary artery acceleration time (PAAT) and the ratio of PAAT over ejection time.⁷³ RV function can be assessed using tricuspid annular plane systolic excursion, RV myocardial performance index, and right ventricular fractional area change, all indicators of pulmonary artery pressure.⁷⁴

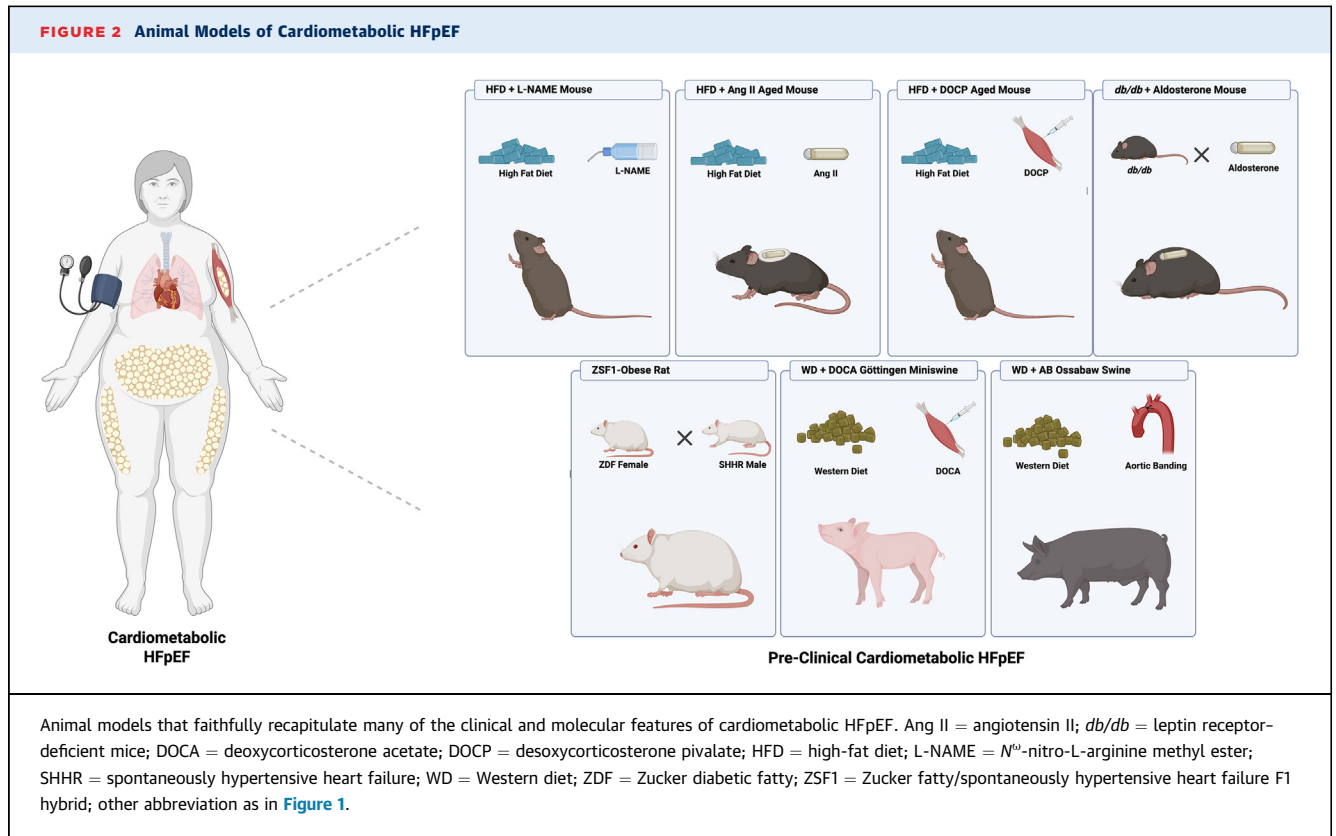
Exercise tolerance can be measured using a motorized treadmill protocol in virtually all animal

species including rodents and pigs.⁷⁵ Additionally, wheel running, and swimming can be performed in rodent species.⁷⁵ Graded exercise tolerance until exhaustion is typically used to measure endurance capacity and is a good indicator of exercise capacity. Generally considered the clinical gold standard for measuring exercise tolerance, VO_{2max} can also be obtained.⁷⁵ Running environment including ambient temperature, stress due to handling, circadian time, and acclimatization to the treadmill can affect results.⁷⁵ Mounting evidence points to skeletal muscle impairment and sarcopenia being contributors to reduced exercise capacity in HFpEF.^{76,77} Assessment of skeletal muscle function, as well as skeletal muscle mass, composition, and oxidative capacity are encouraged.

CKD is prevalent among HFpEF patients and measuring renal function in preclinical models is encouraged. Various measures of renal function include urine protein/creatinine ratio, urine albumin/creatinine ratio, urine N-acetyl- β -(d)-glucosaminidase/creatinine ratio, plasma creatinine, and cystatin-c levels. Invasive techniques can be used to measure renal hemodynamics and glomerular filtration rate. Kidney weight and histopathologic studies are useful supporting studies.

Atrial fibrillation occurs in up to two-thirds of HFpEF patients and is a strong predictor of poor outcomes.⁷⁸ Whereas rodents rarely develop spontaneous atrial fibrillation, rodent HFpEF models manifest increased susceptibility to sustained atrial fibrillation after electrical stimulation and exhibit left atrial remodeling and reduced contractility.⁷⁹ Assessment of the left atria using echocardiography and left atrial arrhythmia inducibility is encouraged.

Cellular and molecular phenotyping. Testing for the characteristic cellular and molecular features of myocardial remodeling is important when characterizing any animal model of cardiometabolic HFpEF. Typical features of HF including cardiac hypertrophy and fibrosis should be assessed by reporting heart weight normalized to tibia length, as well as assessing histopathologic changes and alterations in the expression of molecular markers of hypertrophy and fibrosis. Myocardial stiffness is a characteristic feature of HFpEF which can arise due to functional changes at the level of the cardiomyocyte including actin-myosin interactions, as well as physical changes such as alterations in extracellular matrix composition. Various in vitro and in vivo techniques can be used to assess myocardial stiffness.⁸⁰ Although beyond the scope of this review, assays to gauge myocardial and systemic inflammation, myocardial



energetic dysfunction, mitochondrial and metabolic alterations, oxidative stress, vascular dysfunction, and myocardial steatosis can be performed.

PRECLINICAL MODELS. Based on the 4 features of cardiometabolic HFpEF outlined above, we scored a variety of commonly used HFpEF models on their similarity to human disease ([Table 1](#), [Figure 2](#)). Models that replicate cardiometabolic HFpEF most consistently are discussed in further detail.

ZSF1-obese rats. ZSF1 obese rats display multiple features typical of clinical HFpEF and have been used extensively to study HFpEF preclinically.⁸¹⁻⁸⁴ These rats are obtained by crossing female Zucker Diabetic Fatty rats and male Spontaneously Hypertensive Heart Failure rats.^{85,86} By 10 to 20 weeks of age, both male and female ZSF1 rats develop cardiometabolic traits including obesity, dyslipidemia, insulin resistance, and mild hypertension ([Table 2](#)). Diastolic dysfunction is prominent along with characteristic features of clinical HF, including lung edema and impaired exercise tolerance.^{81,82} Diastolic dysfunction assessed by echocardiography or invasive hemodynamic recordings reveal elevated E/e' ratios and left ventricular end-diastolic pressure, respectively.^{84,87} Whereas EF is preserved, depressed GLS is observed, consistent with clinical HFpEF.²⁷

Concentric myocardial hypertrophy and interstitial fibrosis are reported along with increased cardiomyocyte stiffness attributed to titin post-translational modifications.^{82,88} Macrovascular, myocardial microvascular, and endothelial dysfunction occur along with endothelial nitric oxide synthase (eNOS) uncoupling and reduced protein kinase G (PKG) activity.⁸⁹⁻⁹¹ Skeletal muscle dysfunction, including diaphragm inflexibility, can occur.^{84,92,93} Renal dysfunction develops in ZSF1 rats with evident proteinuria, glycosuria, renal hypertrophy, collagen IV deposition, and resulting fibrosis.^{89,94} Whereas no pulmonary hypertension (PH) is present, the vascular endothelial growth factor receptor blocker (SU5416) has been used to induce PH in this model.^{95,96}

Limitations. Genetic manipulation of the leptin receptor is used to elicit obesity.

High-fat diet + *N*^o-nitro-L-arginine methyl ester “two-hit” mouse. A two-hit approach involving metabolic/inflammatory stress (obesity and metabolic syndrome) coupled with hemodynamic overload (hypertension) has been used to induce cardiometabolic HFpEF in C57BL/6 mice.⁹⁷ Treatment combines 60% high-fat diet (HFD) to induce obesity and metabolic syndrome and an inhibitor of constitutive nitric

TABLE 2 Characteristics of Preclinical Models of Cardiometabolic HFpEF

| Model | ZSF1 Obese Rats | HFD + L-NAME Mouse | db/db + Aldosterone Mouse | HFD + DOCP 22Aged Mouse | HFD+ Ang II Aged Mouse | WD + DOCA Göttingen Miniswine | WD + AB Ossabaw Swine |
|--------------------------------------|-----------------------|--------------------------|---------------------------------|-------------------------------|---------------------------|-------------------------------------|-----------------------------|
| Clinical characteristics | | | | | | | |
| Obesity | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Glucose intolerance | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Hypertension | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Pulmonary congestion | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Pulmonary hypertension | inducible | N/A | N/A | N/A | N/A | ✓ | N/A |
| Exercise intolerance | ✓ | ✓ | N/A | ✓ | N/A | N/A | N/A |
| Atrial remodeling | ✓ | ✓ | ✓ | N/A | ✓ | ✓ | N/A |
| Renal dysfunction | ✓ | N/A | N/A | N/A | ✓ | ✓ | N/A |
| Sarcopenia | ✓ | N/A | N/A | N/A | N/A | N/A | N/A |
| Cardiac functional parameters | | | | | | | |
| Diastolic dysfunction | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Preserved LVEF | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Impaired systolic strain | ✓ | ✓ | N/A | N/A | ✓ | N/A | N/A |
| Impaired cardiac reserve | ✓ | N/A | N/A | N/A | N/A | N/A | N/A |
| Molecular characteristics | | | | | | | |
| Inflammation | ✓ | ✓ | ✓ | ✓ | ✓ | N/A | ✓ |
| Fibrosis | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Cardiomyocyte hypertrophy | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Cardiomyocyte stiffness | ✓ | ✓ | N/A | N/A | N/A | N/A | ✓ |
| Insulin resistance | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Steatosis | ✓ | ✓ | N/A | N/A | N/A | N/A | N/A |
| Vascular dysfunction | ✓ | N/A | N/A | N/A | N/A | ✓ | N/A |
| Oxidative stress | ✓ | N/A | N/A | N/A | N/A | ✓ | N/A |
| Mitochondrial dysfunction | ✓ | ✓ | N/A | ✓ | ✓ | N/A | ✓ |

AB = surgical aortic banding; DOCP = desoxycorticosterone pivalate; LVEF = left ventricular ejection fraction; WD = Western diet; other abbreviations as in Table 1.

oxide synthases, N^G -nitro-L-arginine methyl ester (L-NAME), to induce mild hypertension. This mouse model manifests obesity, insulin resistance, mild hypertension, and faithfully recapitulates many, even most, aspects of clinical cardiometabolic HFpEF.

Echocardiographic assessment reveals diastolic dysfunction, preserved systolic function measured as EF, and reduced GLS. Elevated LV filling pressures are detectable on invasive hemodynamics recordings. Exercise intolerance, pulmonary congestion, and a predisposition to atrial fibrillation are present.⁷⁹ Modest cardiomyocyte hypertrophy, mild interstitial fibrosis, low-grade inflammation, an impaired unfolded protein response, myocardial steatosis, and mitochondrial dysfunction are reported.⁹⁷⁻⁹⁹ Capillary rarefaction and microvascular dysfunction are also present.⁹⁷ Besides metabolic and hypertensive stresses, a “third hit” including an additional HFpEF comorbidity—aging—has been tested in murine models as with the following 2 models.

Limitations. The impact of murine genetic strain differences is unknown. That said, both C57Bl/6J and C57Bl/6N mouse strains develop the HFpEF phenotype as do multiple strains.^{100,101}

HFD + desoxycorticosterone pivalate aged mouse. Three-month-old C57Bl/6J mice were exposed for 13 months to 60% HFD diet and 3 months of desoxycorticosterone pivalate-induced hypertension manifesting several of the main features of cardiometabolic HFpEF.¹⁰² Along with cardiometabolic comorbidities of obesity, hypertension, and glucose intolerance, mice develop diastolic dysfunction with a preserved EF as measured by echocardiography and invasive hemodynamics, exercise intolerance, and pulmonary edema.¹⁰² Cardiac hypertrophy and fibrosis were observed along with impairment of endothelium-dependent vasorelaxation, oxidative stress, and inflammation. Metabolic alterations and mitochondrial dysfunction are also prominent features.

Limitations. Length of treatment is potentially prohibitive. Few published studies have used this model so far.

HFD + angiotensin II aged mouse. Here, cardiometabolic and hypertensive comorbidities were combined with aging to produce a HFpEF phenotype. Eighteen- to 22-month-old C57Bl/6J mice were treated with a 60% HFD diet for 12 weeks and

1.25 mg/kg/d of angiotensin II (Ang II) infused over 4 weeks via an osmotic minipump. These mice develop obesity, hypertension, and glucose intolerance while manifesting various features of cardiometabolic HFpEF including diastolic dysfunction with a preserved EF, atrial enlargement, and lung congestion. These mice develop cardiac hypertrophy and fibrosis, while also manifesting an increase in inflammation and metabolic dysfunction.¹⁰³

Limitations. Ang II is used as a second “hit” whereas renin-angiotensin-aldosterone system blockade has not shown benefit in HFpEF clinical trials.^{31,33,104-107} Ang II at higher doses leads to overt systolic dysfunction. Few published studies have used this model so far.

db/db + aldosterone mouse. Leptin receptor-deficient mice (*db/db*) and leptin-deficient mice (*ob/ob*) have long been used to study diastolic dysfunction. Without an additional hit, both models spontaneously develop obesity, insulin resistance, concentric cardiac hypertrophy and diastolic dysfunction. However, they do not develop pulmonary congestion, an important feature of HFpEF, therefore limiting how representative they are of human disease. In addition, reversal of cardiac remodeling in *ob/ob* mice is observed with infusion of recombinant leptin, with evidence pointing to the loss of leptin-mediated signaling being the driver of remodeling in this model.^{108,109} There is little evidence pointing to that being the case in human cardiometabolic HFpEF.

Exposing 12-week-old *db/db* mice to a second hit by infusion of 7.2 µg/d of aldosterone via an osmotic pump more accurately recapitulates human cardiometabolic HFpEF.¹¹⁰ In addition to the obesity and glucose intolerance manifested in *db/db* mice alone, these mice also develop hypertension. Diastolic dysfunction with a preserved EF and left atrial remodeling can be detected on echocardiography, and notably these mice develop pulmonary congestion. At the cardiomyocyte level, hypertrophy is observed along with arrhythmogenic action potential remodeling.¹¹⁰

Limitations. Genetic manipulation of the leptin receptor is used to elicit obesity. Aldosterone is used as a second hit; however, aldosterone antagonism has not shown benefit in HFpEF.³¹ Few studies have used this model so far.

Western diet + deoxycorticosterone acetate Göttingen miniswine. Using a different two-hit strategy, 14-month-old Göttingen minipigs were exposed to 20 weeks of Western diet (WD) high in fat, fructose, cholesterol, and salt coupled with 16 weeks

of deoxycorticosterone acetate to induce hypertension.¹¹¹ Cardiometabolic comorbidities emerged, including obesity, insulin resistance, hyperlipidemia, and hypertension. Phenotyping studies revealed a preserved EF, diastolic dysfunction, and increased ventricular filling pressures. Atrial remodeling and dysfunction along with PH were evident. Significant myocardial concentric hypertrophy and fibrosis, impaired coronary artery endothelial function, and oxidative stress are features of this model which also manifests renal fibrosis.

Limitations. Cost and facilities required are prohibitive for widespread use.

WD + aortic banded Ossabaw swine. HFpEF was induced over 10 months by feeding 2-month-old Ossabaw swine a WD high in fat, high-fructose corn syrup, and cholesterol while inducing pressure overload with surgical aortic banding (AB).¹¹² In total, animals received 10 months of WD and 6 months of AB. Diastolic function was characterized using invasive pressure measurements revealing an increased end-diastolic pressure volume relationship while systolic function, measured as EF by echocardiography, was preserved. Pulmonary congestion and LV hypertrophy were present.¹¹²

Ossabaw swine on a WD developed features of metabolic syndrome including central obesity, insulin resistance, impaired glucose tolerance, dyslipidemia, and hypertension.¹¹³ WD + AB animals developed significant cardiac hypertrophy and manifested evidence of cardiomyocyte contractile dysfunction, elevated fibrosis markers, low-grade inflammation, mitochondrial dysfunction, and titin isoform shifts.¹¹²

Limitations. Cost and facilities required are prohibitive for widespread use. Surgical intervention is technically difficult, and AB can lead to overt systolic dysfunction if done improperly.

MOLECULAR MECHANISMS OF CARDIOMETABOLIC HFpEF

CARDIOMYOCYTE HYPERTROPHY AND STIFFNESS.

Cardiomyocyte hypertrophy and stiffness are central elements in mediating diastolic dysfunction in cardiometabolic HFpEF. Cardiomyocytes isolated from HFpEF endomyocardial biopsies display increased resting tension, a finding absent in HFrEF.¹¹⁴⁻¹¹⁶ Cardiometabolic comorbidities seem to influence cardiomyocyte stiffness in which diabetes was shown to increase cardiomyocyte resting tension in patients with HFpEF.¹¹⁴ Animal models of cardiometabolic HFpEF display hypertrophy and stiffness at the cardiomyocyte level.^{82,97,112}

Modification of components of the thin and thick filaments, including titin, troponin I, myosin binding protein C, tropomyosin, and myosin heavy chain are crucial regulators of cardiomyocyte distensibility and compliance.^{117,118} Titin compliance is primarily determined by titin post-translational modifications and isoform shifts and has received special attention in HFpEF.^{83,119-123} Titin hypophosphorylation of N2B and hyperphosphorylation of proline-glutamate-valine-lysine (PEVK) segments have been reported in human HFpEF endomyocardial biopsies whereas hypophosphorylation of elastic N2Bus and PEVK were found to mediate myocardial stiffness in ZSF1 rats.^{82,124} These findings have been linked to reduced PKG activity which is held to result from comorbidity-driven endothelial inflammation and subsequent dysregulation of the eNOS-cyclic guanosine monophosphate-PKG axis.^{83,90,125} Treatment with the sodium-glucose cotransporter 2 inhibitor empagliflozin is associated with increases in the phosphorylation of troponin I, myosin binding protein C, and titin, increasing myocardial compliance in HFpEF patients.¹²⁶ Metformin has a similar effect increasing N2B phosphorylation.¹²⁷ Myofilament acetylation also affects chamber compliance and diastolic function. HDAC6 increases titin distensibility via PEVK segment deacetylation whereas troponin I acetylation increases relaxation time in cardiomyocytes.^{128,129} Whereas no changes in titin acetylation were detected in obese ZSF1 rats, *in vitro* deacetylation of stiff N2B titin in isolated cardiomyocytes, using sirtuin 1 (SIRT1), reduces cardiomyocyte passive tension.¹³⁰

Cardiomyocytes in the adult heart harbor 2 isoforms of titin, N2B and N2BA, the former being shorter and less compliant. A higher N2BA:N2B ratio, favoring increased myocardial compliance, has been described in ischemic and nonischemic dilated cardiomyopathy.¹³¹⁻¹³³ Whereas no titin isoform shifts have been described in HFpEF, upregulating compliant titin isoforms via inhibition of splicing factor RNA-binding motif 20 improves diastolic dysfunction in some genetic models.^{134,135} Higher serum levels of a matrix-metalloproteinase-12 cleaved fragment of titin are associated with worse outcomes in HFpEF, making it a potential biomarker of disease severity.¹³⁶ Abundance and post-translational modification of cytoskeletal α -tubulin have also been implicated in ZSF1 rats.¹³⁷

Cardiomyocyte hypertrophy is a cardinal feature of HFpEF and a finding present in all above-mentioned preclinical models.¹³⁸ Cardiomyocyte hypertrophy has been extensively studied in various preclinical models of HF. A wide range of molecular pathways have been implicated in maladaptive cardiomyocyte hypertrophy and may be conserved in

cardiometabolic HFpEF. The major drivers of cardiomyocyte hypertrophy include neurohormonal signals (eg, Ang II, catecholamines, endothelin I) and mechanical stress, all of which alter a plethora of downstream pathways and contribute to reactive oxygen species generation, inflammation, metabolic alterations, fibrosis, and a host of other pathogenic changes.¹³⁹ Whereas beta-blockers and Ang II antagonism have not yielded positive effects in HFpEF, the PARAGON-HF (Efficacy and Safety of LCZ696 Compared to Valsartan, on Morbidity and Mortality in Heart Failure Patients With Preserved Ejection Fraction) trial using angiotensin receptor/neprilysin inhibition in HFpEF indicated that there may be some benefit in women and those with lower EFs.³³

Epigenetic modifications such as DNA methylation, histone modifications, and noncoding RNAs are strongly implicated in cardiomyocyte hypertrophy and are likely to play a significant role in regulating cardiomyocyte hypertrophy and other pathogenic processes in cardiometabolic HFpEF.¹⁴⁰ The genetics of HFpEF also remain understudied as few genome-wide association studies have investigated the genetic basis of the syndrome. One study identified only a single locus associated with HFpEF, in stark contrast to the 13 loci identified for HFrEF, highlighting the genetic complexity and heterogeneity of the HFpEF population.¹⁴¹

INFLAMMATION AND EXTRACELLULAR MATRIX REMODELING. Cardiometabolic HFpEF is associated with chronic low-grade inflammation at both systemic and myocardial levels.^{142,143} The most prevalent comorbidities associated with cardiometabolic HFpEF, including obesity, diabetes, hypertension, and aging, are all characterized by states of chronic low-grade inflammation.^{142,144-149} The conglomeration of these comorbidities is thought to drive inflammation in HFpEF, and evidence continues to emerge implicating comorbidity-driven inflammation in various HFpEF pathophysiological mechanisms. Additional evidence for the comorbidity-inflammation paradigm can be drawn from proteomics mediation analysis performed from the PROMIS-HFpEF (Prevalence and correlates of coronary microvascular dysfunction in heart failure with preserved ejection fraction) study indicating that inflammation mediates the association between comorbidity burden and worsened myocardial structure and diastolic dysfunction in HFpEF, with several proteins identified as strong mediators of this association.¹⁵⁰

Chronic low-grade inflammation is noted in animal models of cardiometabolic HFpEF and can accentuate various pathologic findings, including cardiomyocyte hypertrophy and stiffness, extracellular matrix

remodeling, oxidative/nitrosative stress, and mitochondrial and metabolic dysfunction.^{142,151} As we have shown, in the HFD + L-NAME mouse and human HFpEF, upregulation of inducible nitric oxide synthase (iNOS), a downstream mediator of inflammation, and subsequent S-nitrosylation of the endonuclease inositol-requiring protein 1 α disrupts Xbp1 splicing, leading to a diminished ability to handle endoplasmic reticulum stress with subsequent cardiomyocyte dysfunction.⁹⁷ It is important to recognize that L-NAME used in the generation of this model is an inhibitor of the constitutive nitric oxide synthases and neuronal nitric oxide synthase (nNOS), and does not impact iNOS activity with a dissociation constant that is 20-fold higher.^{152,153} Furthermore, nitrosative/oxidative stress is thought to drive endothelial inflammation and the defective eNOS-cyclic guanosine monophosphate-PKG axis in HFpEF.¹⁵⁴ Inflammation can also mediate profibrotic LV remodeling in which immune cell-fibroblast crosstalk drives activation and myofibroblast transformation.¹⁵⁵

In the context of obesity observed in cardiometabolic HFpEF, “meta-inflammation” or “metabolic inflammation,” the process whereby obesity-mediated metabolic stress drives inflammation, is emerging as a driver of syndrome pathogenesis.¹⁵⁶ Adipose tissue expansion and inflammation can contribute to the increased circulating cytokines observed in obesity and HFpEF and can modulate myocardial remodeling.^{149,151,157-161} Weight loss has been shown to reduce inflammation and improve myocardial remodeling in HFpEF patients.³⁴ Different adipose tissue depots may play specific roles in HFpEF, with an especially important role suggested for epicardial adipose tissue (EAT) due its proximity and interconnection with the myocardium.¹⁶² Increased EAT is observed in both cardiometabolic and non-cardiometabolic HFpEF compared with healthy controls despite a similar BMI.³⁸ EAT expansion in HFpEF has been associated with worse hemodynamic parameters, impaired exercise capacity, increased HF hospitalizations, and higher all-cause mortality.^{163,164} Mechanistically, increased EAT is associated with myocardial remodeling, endothelial dysfunction, systemic inflammation, and insulin resistance in HFpEF patients.¹⁶⁵ EAT can act as a reservoir for various immune cell populations while also secreting inflammatory cytokines and adipokines that affect both systemic and myocardial inflammation.^{159,166,167} EAT can mediate cardiomyocyte hypertrophy and is metabolically active allowing it to increase myocardial insulin resistance via secretion of retinol-binding protein 4, promote myocardial steatosis, and even affect systemic metabolism.^{158,161,168,169}

Extracellular matrix remodeling governed by cardiac fibroblasts can drive myocardial interstitial fibrosis altering diastolic function.^{151,170} Some level of myocardial fibrosis is observed in obesity, metabolic syndrome, clinical HFpEF, and across all preclinical models of cardiometabolic HFpEF.¹⁷¹⁻¹⁷⁵ A vast array of triggers and pathways govern fibroblast activation.^{170,176} Fibroblast crosstalk with immune cells and other tissues is crucial in mediating increased fibrosis. For example, visceral adipose tissue-secreted osteopontin can modulate profibrotic transformation of cardiac fibroblasts contributing to myocardial fibrosis and dysfunction.¹⁶⁰ True to the multisystemic nature of cardiometabolic HFpEF, liver-heart crosstalk via coagulation factor XI was shown to protect against HFpEF by activating the bone morphogenetic protein-SMAD 1/5 pathway in the heart.¹⁷⁷⁻¹⁷⁹ Endothelial to mesenchymal transition may also play a significant role in cardiac fibrosis.¹⁸⁰

MITOCHONDRIAL DYSFUNCTION AND METABOLIC PERTURBATIONS. Metabolic perturbations and mitochondrial dysfunction observed in HFpEF have far reaching consequences on cardiomyocyte energy status and are posited to play a significant role in syndrome pathogenesis. Reduced cardiac energy reserves measured as cardiac creatine phosphate to adenine triphosphate (ATP) ratio are a feature of both HFpEF and HFrEF.¹⁸¹ Obesity alone results in decreased cardiac phosphocreatine/ATP ratios, with ATP production compensated at rest, but not during exercise.¹⁸²

Metabolic stress is accompanied by cardiac metabolic inflexibility and altered substrate use.^{98,183,184} However, with respect to HFpEF, our understanding of the metabolic remodeling mechanisms remains at a very early stage. Human HFpEF cardiac samples are scarce and most currently available data are extrapolated from myocardial biopsies obtained from the RV in limited clinical centers with inconsistent fasting status. Whereas reduced cardiac glucose use and increased long-chain fatty acid uptake and oxidation are characteristic features of cardiac metabolism under conditions of metabolic stress, recent evidence suggests myocardial metabolism in cardiometabolic HFpEF has unique features.^{185,186} Metabolomics analyses performed on endomyocardial biopsies from HFpEF, HFrEF, and healthy controls reveal. 1) Genes and metabolites of fatty acid oxidation pathways are reduced in HFpEF as compared with healthy controls, 2) Glucose metabolism-related gene expression is reduced in HFpEF relative to healthy controls. Pyruvate levels are higher and tricarboxylic acid cycle

intermediates are lower in HFpEF, 3) Levels of branched chain amino acids (BCAAs) are higher in HFpEF. Genes involved in BCAA catabolism and BCAA catabolites are lower implying impaired catabolism, and 4) Levels of the ketone beta-hydroxybutyric acid are lower in HFpEF.¹⁸⁷

To directly assess cardiac substrate use, O'Sullivan et al¹⁸⁸ performed metabolomics and lipidomics analyses in arterial and coronary sinus blood samples obtained from nonfasted HFpEF patients and healthy controls. They discovered that HFpEF hearts have impaired uptake of free fatty acids and glucose, but with a concomitant transition to more complex lipid generation and protein catabolism. No significant differences were observed in cardiac extraction of BCAA or ketone bodies. Based on these studies, cardiometabolic HFpEF hearts seem to display impairment in the usage of multiple substrates with consequent fuel inflexibility.

Metabolic pathways have also been studied in various preclinical models. Whereas maximal glucose and fatty acid oxidation are impaired in mitochondria isolated from the HFD + L-NAME mouse hearts, a recent ex vivo flux study suggested that fatty acids are still the major substrate whereas insulin-mediated glucose oxidation is severely impaired and glycolysis and ketone oxidation remain unchanged.¹⁸⁹ Additional studies are needed to better elucidate cardiometabolic HFpEF-associated metabolic remodeling and how it differs from obesity alone.

Mitochondrial architecture and oxidative capacity are altered in obesity and cardiometabolic HFpEF.^{98,100,190,191} Whereas mechanisms remain understudied, various factors may drive mitochondrial dysfunction: (1) Altered mitochondrial protein expression and post-translational modifications.¹⁸³ Mitochondrial protein hyperacetylation due to SIRT3 downregulation is a feature of cardiometabolic HFpEF and may drive metabolic dysregulation and inflammation.^{98,102,192} Proteins involved in oxidative phosphorylation, the tricarboxylic acid cycle and fatty acid oxidation are especially susceptible to hyperacetylation.¹⁹² The exact implication of this post-translational modification remains poorly understood and controversial.¹⁹³ (2) Impairments in mitochondrial biogenesis, mitochondrial dynamics, and mitophagy have been reported.¹⁹⁴ (3) Dysregulation of mitochondrial Ca²⁺ homeostasis is a finding observed in ZSF1 rats.^{195,196} (4) Increased reactive oxygen species has been described, driven largely by the mitochondrial respiratory chain and other mitochondrial proteins, such as nicotinamide adenine dinucleotide phosphate oxidase (nicotinamide

adenine dinucleotide phosphate oxidase 2/4), cytochrome P450 enzymes, and xanthine oxidase.¹⁹⁷

Various strategies have targeted mitochondrial dysfunction and metabolic changes in cardiometabolic HFpEF. Encouraging results from the EMPEROR-Preserved (Empagliflozin in Heart Failure with a Preserved Ejection Fraction) trial revealed that empagliflozin can reduce HF hospitalizations in HFpEF patients.³² Favorable metabolic alterations induced by sodium-glucose cotransporter 2 inhibitors include increasing ketone availability and altering mitochondrial number, structure, and dynamics.¹⁹⁸⁻²⁰¹ Reduced expression of nicotinamide phosphoribosyltransferase disrupts the nicotinamide adenine dinucleotide (NAD) salvage pathway triggering a reduced NAD⁺/nicotinamide adenine dinucleotide, hydrogen (NADH) ratio seen across various models of cardiometabolic HFpEF.^{98,130,192} Supplementation with nicotinamide riboside, an NAD⁺ precursor, reduces mitochondrial hyperacetylation, improves mitochondrial function, and ameliorates the HFpEF phenotype.^{98,130,192} Indole-3-propionic acid, a metabolite produced by gut microbiota, is reduced in both the L-NAME + HFD mouse model and in human HFpEF cohorts.²⁰² Supplementing indole-3-propionic acid mediates protective effects in HFpEF by promoting the expression of SIRT3 and augmenting NAD⁺/NADH ratio.²⁰² Overexpression of acyl-coenzyme A synthetase long chain family member 6 protects against mitochondrial dysfunction in cardiometabolic HFpEF.¹⁰⁰ Furthermore, supplementation with beta-hydroxybutyric acid reverses cardiometabolic HFpEF by inducing a host of favorable metabolic changes and inhibiting mitochondrial NOD-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome assembly.¹⁰²

INSULIN RESISTANCE. A characteristic feature of obesity and diabetes—insulin resistance—is prevalent in HF, including cardiometabolic HFpEF, and is observed across all preclinical models discussed here. Insulin resistance mediates a host of downstream alterations in the metabolic milieu, including meta-inflammatory changes that have far reaching systemic and myocardial consequences.²⁰³

Insulin binds to insulin receptors or the insulin-like-growth-factor 1 receptor activating IRS1/2, PI3K, Akt, and mitogen-activated protein kinases that promote GLUT4 membrane translocation and regulate a host of downstream cellular processes including cell growth and survival, metabolism, and autophagy.^{204,205} PI3K/Akt signaling regulates a host of processes including cardiomyocyte growth, autophagy, metabolism, and cell death.²⁰⁶⁻²¹⁰ Insulin resistance entails reduced ability of insulin to promote glucose uptake and elicits other downstream

effects. The metabolic milieu in insulin resistance is characterized by increases in circulating triglycerides, fatty acids, and glucose. Increased circulating inflammatory cytokines, catecholamines, and growth hormones are also characteristic. These changes are accompanied by mitochondrial dysfunction, oxidative stress, and endoplasmic reticulum stress.

Although little work has been performed to examine pathways affected by insulin resistance in bona fide models of cardiometabolic HFpEF, various points can be inferred from preclinical models of metabolic syndrome. Models of severe insulin resistance, including *ob/ob* mice, or mice fed 60% fat diet, display impaired Akt activation, reduced GLUT4 localization to the plasma membrane, and increased FoxO1 nuclear translocation and activation in the heart.^{185,211,212} In the HFD + L-NAME mouse model, increased FoxO1 activation occurs in the setting of reduced Xbp1s, leading to cardiomyocyte lipid accumulation. Both FoxO1 depletion and Xbp1s overexpression are sufficient to elicit HFpEF reversal in this model.⁹⁹ These findings highlight the importance of the Xbp1s-FoxO1 axis in HFpEF pathogenesis. However, severity and duration of insulin resistance seem to affect these pathways differently.²⁰³ In mice exposed to a 45% fat diet for 2 weeks, a milder model of insulin resistance, the Akt pathway, is preserved and, in some cases, further activated despite reduced GLUT4 plasma membrane localization.²¹³ Findings from endomyocardial biopsies harvested from diabetic or HFrEF patients also indicate that severity of disease may affect the state of the PI3K/Akt pathways.^{214,215} However, both excessive or reduced activation of Akt may contribute to myocardial remodeling and disease pathogenesis at different stages of HF.^{203,207,216,217}

AMPK is a regulator of contraction-induced GLUT4 translocation in cardiomyocytes, governs a wide range of vital cellular processes, and impaired activation plays a pathogenic role in insulin resistance, metabolic syndrome, and cardiometabolic HFpEF.^{79,218-221} Impaired AMPK activation mediates HFpEF-associated atrial fibrillation in the HFD + L-NAME mouse model.⁷⁹ Activation of AMPK can increase insulin sensitivity systemically and improve atrial remodeling observed in HFpEF.^{79,222} Therapies improving insulin resistance may be of added benefit in HFpEF patients; however, caution is warranted as some diabetes drugs, such as thiazolidines and the dipeptidyl peptidase-4 inhibitor saxagliptin, may increase HF hospitalizations.^{223,224}

SKELETAL MUSCLE IMPAIRMENT. Exercise intolerance is a cardinal feature of HFpEF measured as

decreased VO_{2max} . Although exercise intolerance has traditionally been attributed to cardiac dysfunction, evidence points to extracardiac factors, especially skeletal muscle alterations as critical contributors to reduced exercise capacity. Sarcopenia and severe skeletal muscle impairment seem specific to HFpEF as opposed to other types of HF; HFpEF patients manifest worse muscle function, reduced mitochondrial size, and increased expression of atrophy-related genes when compared with HFrEF patients or healthy controls.⁷⁶ Respirometry assays performed on skeletal muscle biopsies from HFpEF patients also reveal impaired mitochondrial function.²²⁵ Weight loss in HFD-fed mice increases skeletal muscle mitochondrial efficiency.²²⁶ Increased intramuscular fat has also been observed in HFpEF patients; this altered skeletal muscle composition was related to the reduced VO_2 observed.²²⁷

Although skeletal muscle changes remain understudied in HFpEF, an imbalance between anabolic and catabolic pathways may contribute to the presence of sarcopenia. Potential mechanisms include: 1) blunting of the IGF-PI3K-Akt pathway, inhibiting protein synthesis and promoting atrophy in HFpEF^{76,228,229}; 2) increased circulating proinflammatory cytokines, including myostatin, which are known triggers of skeletal muscle atrophy^{76,230}; 3) altered skeletal muscle composition such as increased intramuscular fat and altered slow/fast twitch fiber composition²²⁷; 4) mitochondrial dysfunction; 5) activation of autophagy and the ubiquitin-proteasome system promoting protein degradation^{231,232}; 6) increased skeletal muscle apoptosis²³³; and 7) activation of the sympathetic nervous and renin-angiotensin-aldosterone axis.^{234,235}

CONCLUSIONS

Emerging evidence indicates that cardiometabolic HFpEF is a distinct, and the most prevalent, endotype of HFpEF. This recognition is crucial, as it underscores the need for tailored therapeutic strategies that specifically address the unique pathophysiological mechanisms associated with cardiometabolic HFpEF and its metabolic comorbidities. By focusing on the distinct characteristics of cardiometabolic HFpEF, investigators and clinicians can develop more effective therapeutic interventions. As we continue to unravel the complexities of this HFpEF endotype, it becomes increasingly evident that a deeper understanding of cardiometabolic HFpEF is only possible by studying patient cohorts and preclinical animal models that accurately reflect the clinical realities of the syndrome.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

This work was supported by grants from the NIH: HL-128215 (Dr. Hill), HL-147933 (Dr. Hill), HL-155765 (Dr. Hill, Dr. Gillette), HL-164586 (Dr. Hill, Dr. Gillette), S10RR023729 (Dr. Hill), HL157697 (Dr. Tong); AHA 24 TPA1297904 (Dr. Tong) and DZHK (German Centre for Cardiovascular Research - 81X3100210; 81X2100282) (Dr. Schiattarella), the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation - SFB-1470-A02; SFB-1470-Z01) (Dr. Schiattarella), and the European Research Council - ERC StG 101078307 (Dr.

Schiattarella). All authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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KEY WORDS cardiometabolic, heart failure with preserved ejection fraction, preclinical models