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STATE OF THE ART REVIEW
Heart failure and cardiomyopathies

Heart failure with preserved ejection fraction in humans and mice: embracing clinical complexity in mouse models

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Heart failure (HF) with preserved ejection fraction (HFpEF) is a multifactorial disease accounting for a large and increasing proportion of all clinical HF presentations. As a clinical syndrome, HFpEF is characterized by typical signs and symptoms of HF, a distinct cardiac phenotype and raised natriuretic peptides. Non-cardiac comorbidities frequently co-exist and contribute to the pathophysiology of HFpEF. To date, no therapy has proven to improve outcomes in HFpEF, with drug development hampered, at least partly, by lack of consensus on appropriate standards for pre-clinical HFpEF models. Recently, two clinical algorithms (HFA-PEFF and H_2FPEF scores) have been developed to improve and standardize the diagnosis of HFpEF. In this review, we evaluate the translational utility of HFpEF mouse models in the context of these HFpEF scores. We systematically recorded evidence of symptoms and signs of HF or clinical HFpEF features and included several cardiac and extra-cardiac parameters as well as age and sex for each HFpEF mouse model. We found that most of the pre-clinical HFpEF models do not meet the HFpEF clinical criteria, although some multifactorial models resemble human HFpEF to a reasonable extent. We therefore conclude that to optimize the translational value of mouse models to human HFpEF, a novel approach for the development of pre-clinical HFpEF models is needed, taking into account the complex HFpEF pathophysiology in humans.

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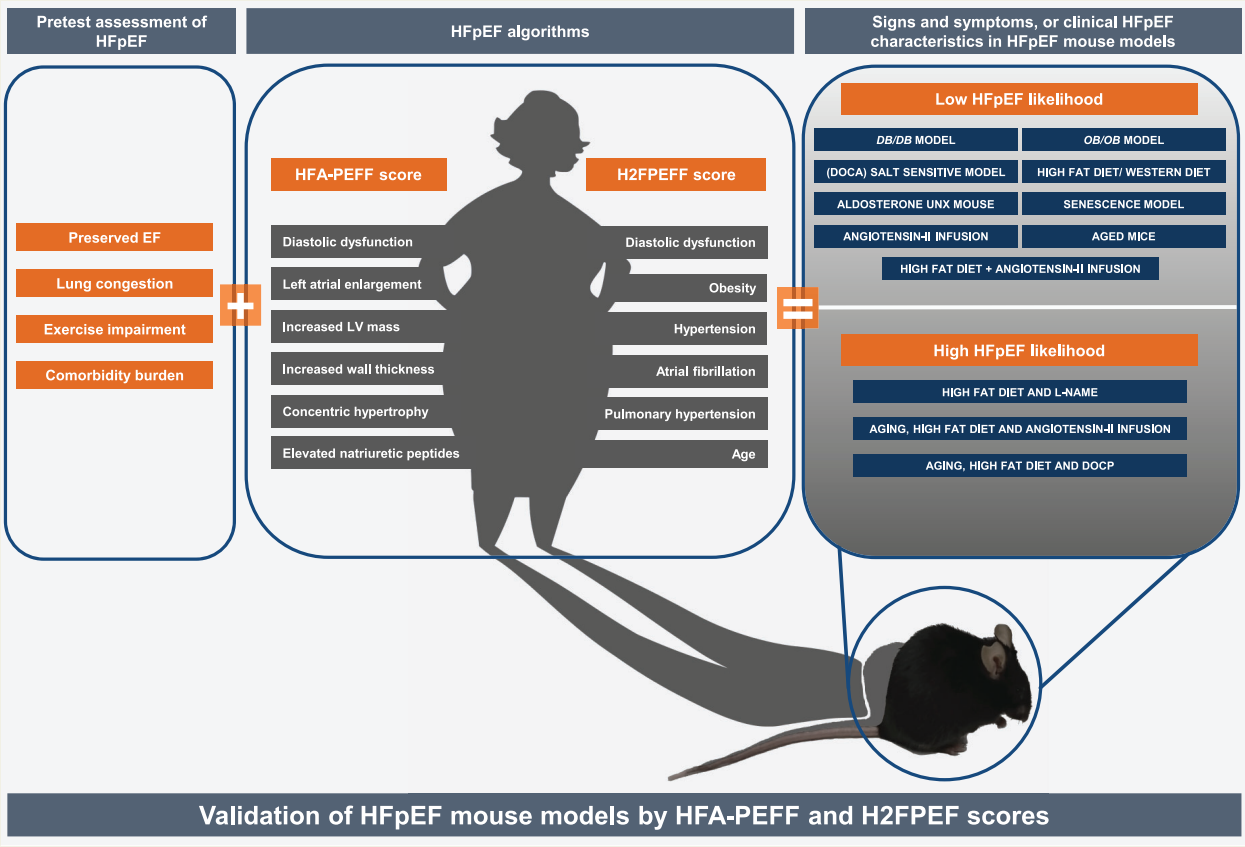
Graphical Abstract



An in-depth review of existing pre-clinical HFpEF mouse models with validation of their translational value using the HFA-PEFF and H2FPEF scores.

Keywords HFpEF • Mouse • Human • Translational • H2FPEF • HFA-PEFF

HFpEF: a heterogeneous disease with multiple disease mechanisms

Heart failure (HF) with preserved ejection fraction (HFpEF) is a complex clinical syndrome that is characterized by both extra-cardiac and cardiac features.[1–3] Prevalence is still rising[4–8] and survival of patients with HFpEF is poor, with a 5-year survival rate after first hospitalization of 35–40%.[9,10] So far no treatment has been proven successful in reducing morbidity and mortality rates in HFpEF, potentially due to the large pathophysiological heterogeneity and diversity in HFpEF phenotypes.[11] Recent studies have identified HFpEF as a systemic disease that is associated with, or may be triggered by a wide range of clinical risk factors and comorbidities such as aging, female sex, hypertension,[12,13] pulmonary congestion, metabolic syndrome, obesity,[7,12,14–16] type 2 diabetes mellitus (T2DM), hyperlipidaemia, renal disease, atrial fibrillation (AF), and skeletal muscle weakness.[11] These risk factors and comorbidities give rise to intertwining disease mechanisms in the pathophysiology of HFpEF.[17,18] Due to the wide range of comorbidities and clinical presentations, potential underlying aetiology of HFpEF is diverse; HFpEF can result from various structural abnormalities of the myocardium, or may result from abnormal loading conditions, e.g. as seen in hypertension, valvular diseases, volume overload, or rhythm disorders.[19]

Although HFpEF patients thus represent a heterogeneous group with a broad extent of extra-cardiac features, the cardiac phenotype has less interpatient variability and includes (concentric) left ventricular (LV) hypertrophy,[20] LV diastolic dysfunction,[21] cardiac stiffening, atrial dilatation, fibrosis,[22] (systemic) inflammation, microvascular endothelial dysfunction,[23,24] and elevated natriuretic peptides.[19,25,26]

The definition of HFpEF as a clinical syndrome, based on typical symptoms and signs, presents challenges due to non-specificity of cardinal symptoms such as breathlessness and effort intolerance. Recently, two diagnostic HFpEF algorithms, the HFA-PEFF[20] and

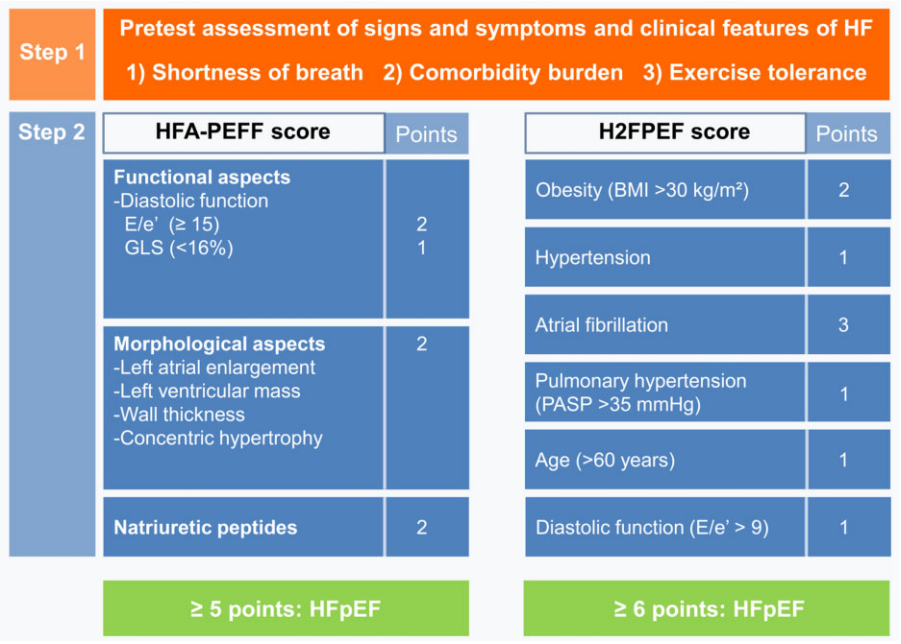


Figure 1 Diagnostic HFpEF scoring algorithms used to score HFpEF animal models. Both algorithms first include a pretest assessment to evaluate signs and symptoms and clinical features of HFpEF that include congestion, increased comorbidity burden and reduced exercise tolerance. The second step of the HFA-PEFF[19] score assesses three domains that include functional aspects [echocardiographic diastolic function (*E*/*e*′ and GLS)], morphological aspects (left atrial enlargement, LV mass and wall thickness and concentric hypertrophy) as well as levels of circulating natriuretic peptides.[2,28] The H_2FPEF[27] score combines clinical and echocardiographic patient characteristics: obesity, hypertension, AF, pulmonary hypertension, age >60 years and diastolic function (*E*/*e*′). A higher score represents a higher likelihood of having HFpEF (HFA-PEFF ≥5 points; H_2FPEF >6 points), while a lower score is used to rule out HFpEF. For patients with an intermediate score, both algorithms recommend additional testing to refine the diagnosis by exercise echocardiography or invasive measurements of cardiac filling pressures in a non-resting state.[19,27] AF, atrial fibrillation; GLS, global longitudinal strain; HF, heart failure; LV, left ventricle; PASP, pulmonary artery systolic pressure.

H_2FPEF[27] scores, were developed to standardize and improve the accuracy of HFpEF diagnosis. Both of these scores (*Figure 1*) use a stepwise diagnostic approach to score and evaluate probability of HFpEF presence. The H_2FPEF score uses functional echocardiographic data and places emphasis on the presence of comorbidities (e.g. hypertension, obesity) and the effect of age, while not including natriuretic peptide levels. The HFA-PEFF algorithm also assesses pretest probability based on clinical features (including age and comorbidities) and similarly includes a score but based on both functional and structural echocardiographic data, including morphological aspects of the left atrium and LV, as well as levels of natriuretic peptides, such as N-terminal pro brain natriuretic peptide (NT-proBNP).

Both HFpEF scores have recently been validated in various patient cohorts[29–33] and communities studies[34] and it was concluded that both HFpEF scores categorized patients well, especially in those patients with intermediate and high scores. These scores, however, are not without controversy, with criticisms ranging from over-simplification of the diagnostic challenges to over-complicating the diagnostic process by requiring expensive tests or the scores largely disagree.[35,36] In addition, misclassification has been reported, especially in those patients with low HFpEF scores, potentially due to the fact that both scores use resting parameters in a phenotype in which physiological abnormalities augment during exercise.[33,37] Nevertheless, both scores have been shown to have prognostic utility in human patients,[38,39] suggesting that they capture key pathophysiologic components that determine outcomes in HFpEF.

Of note, the combined considerations of the phenotypic complexity of HFpEF, the interplay of cardiac and non-cardiac comorbidities, and the role that these comorbidities play in the pathophysiology of HFpEF have not been adequately taken into account in the evaluation of pre-clinical models of HFpEF. While the HFA-PEFF and H_2FPEF algorithms have been developed to standardize and improve HFpEF diagnosis in patients, these scores may represent a novel approach to improve putative applicability of HFpEF mouse models. Therefore, this review aims to evaluate the translational aspects of currently available pre-clinical mouse models of HFpEF in the context of the HFA-PEFF and H_2FPEF scores and proposes a novel approach to the assessment and development of future pre-clinical HFpEF models.

HFpEF in mice: where do we stand?

Over the last decades, development of HFpEF specific treatments has been disappointing. Standard, successful, HF with reduced ejection fraction (HFrEF) treatment options, such as angiotensin-converting enzyme inhibitors (ACEi), angiotensin receptor 1 blockers and mineralocorticoid receptor antagonists (MRA) did not convincingly reduce mortality and morbidity rates in HFpEF patients.[40–42] Trials with other types of drugs, such as nitric oxide donors and cyclic guanosine monophosphate (cGMP) stimulating therapies failed to improve clinical status,[43–47] or were neutral for the primary endpoint (angiotensin receptor–neprilysin inhibitor, PARAGON-HF trial[48,49]). To date, no HFpEF specific treatment options exist and there is an unmet need to improve morbidity and mortality rate in these patients.

Drug development typically progresses in stages, from pre-clinical to clinical. Valuable HFpEF animal models presenting clinical HFpEF phenotypes are crucial for the successful design of new therapies. This has been neglected so far, which has led to the failure of many clinical studies. Sildenafil, for example, successfully reduced LV hypertrophy and cardiac remodelling in mice that suffered from angiotensin II (ANGII)-induced or transverse aortic constriction (TAC) induced HF.[50,51] Clinical studies of sildenafil in HFpEF patients, however, did not observe these beneficial effects on clinical or hemodynamic parameters.[45] Studies with ACEi in myocardial infarction models (MI),[52,53] successfully reduced hypertrophy and fibrosis with a concomitant improvement of cardiac function. However, studies in patients with HFpEF have yielded inconsistent results.[40] This was also the case for the MRA spironolactone: in pre-clinical studies in diet induced[51,54] and myocardial infarction (MI)[55,56] models this drug improved systolic and diastolic cardiac function. A subsequent large randomized controlled trial on the other hand, remained neutral and did not meet its endpoint.[41] The unsuccessful bench-to-bedside translation may, at least partly, be explained by the fact that pre-clinical animals models not fully recapitulate the clinical HFpEF phenotype and TAC or MI models cannot be considered as HFpEF model.

In this review we discuss and score several pre-clinical HFpEF models using the HFA-PEFF and H_2FPEF scores. We found that several major discrepancies exist between pre-clinical HF models and clinical HFpEF. Pre-clinical HFpEF models do not always recognize the importance of signs and symptoms of HFpEF, or clinical HFpEF characteristics (*graphical abstract*). Several so-called HFpEF models would have obtained high scores according to the HFA-PEFF and H_2FPEF risk scores (*Figure 2*) due to functional or morphological features, while signs of lung congestion or exercise impairment were absent and levels of natriuretic peptides low (*Table 1*). Thus, a model without pulmonary congestion may relate to hypertensive heart disease in humans rather than clinical HFpEF (for example *db/db* or *ob/ob* models). The currently developed HFA-PEFF and H_2FPEF scores both emphasize typical symptoms and signs of HF, or clinical HFpEF characteristics as key for the diagnosis of HFpEF. Although the assessment of signs and symptoms or diagnostic HF criteria may be more challenging in animals than in humans, it is not impossible. Pulmonary congestion can be demonstrated by increased lung weight, and reduced exercise tolerance can be measured via voluntary or forced exercise testing. Reduced exercise tolerance is one of the hallmarks in human HFpEF and should ideally be part of phenotyping HFpEF animal models.

Importantly, the demonstration of LV diastolic dysfunction has been the cornerstone of validation of a HFpEF animal model; however, the presence of diastolic dysfunction alone is neither synonymous nor sufficient for a diagnosis of HFpEF. Indeed, diastolic dysfunction, as occurs with aging, can exist without the presence of symptomatic HF. Nonetheless, aging is a potent risk factor for HFpEF.[7,57,58] Aging itself is associated with ventricular-vascular stiffening and fibrosis, key mechanisms in the pathogenesis of HFpEF.[59,60] The aging process also exacerbates chronic systemic inflammation, dysregulation of energy supply[61–63] and increased cardiomyocyte stiffness and increased hypertrophy that may all result in HFpEF specific diastolic dysfunction and cardiac remodelling.[64,65] We realize that aging itself can have major practical limitations (>20 months to produce the phenotype); however, because it is such an important factor, we encourage researchers to include it.

Another major difference between animal and human HFpEF can be found in disease complexity and disease heterogeneity. In humans, HFpEF is considered a multifactorial and heterogeneous disease with a plethora of clinical manifestations.[11] For many years, pre-clinical HFpEF models have relied upon a single perturbation. The development of several recent multifactorial models has shown that it is feasible to develop a HFpEF-like phenotype in mice by using multiple perturbations, and these models may represent a new era of multifactorial pre-clinical HFpEF models.

HFpEF in mice: fundamental checklist

We do not believe that 'one-size-fits-all' pre-clinical HFpEF model exists. Several animal models of HFpEF have been developed that only focused on a limited aspect of this multifactorial syndrome. This strategy has been proven unsuccessful and the recent development of combinatory models is very promising.[66–68] Although recent multifactorial HFpEF models have been proven valuable, and may improve bench-to-bed translation, these models also focus on specific HFpEF phenotypes and do not recapitulate the entire heterogeneity of the clinical HFpEF syndrome. In addition, technical challenges remain in developing mouse models. AF, for example, has not been included in any of the pre-clinical HFpEF models so far.

We therefore suggest that all pre-clinical HFpEF studies should include a mouse model that fulfils (a majority of) the following requirements in order to perform a reliable and accurate pre-clinical HFpEF study. This has been schematically presented in *Figure 3*.

Pretest assessment of signs and symptoms and clinical HFpEF features

First of all, ejection fraction should be preserved. Assessment of symptoms such as shortness of breath, fatigue, oedema, tachycardia, and exercise impairment in animals may be less straightforward than in humans, but various parameters are available to provide a global

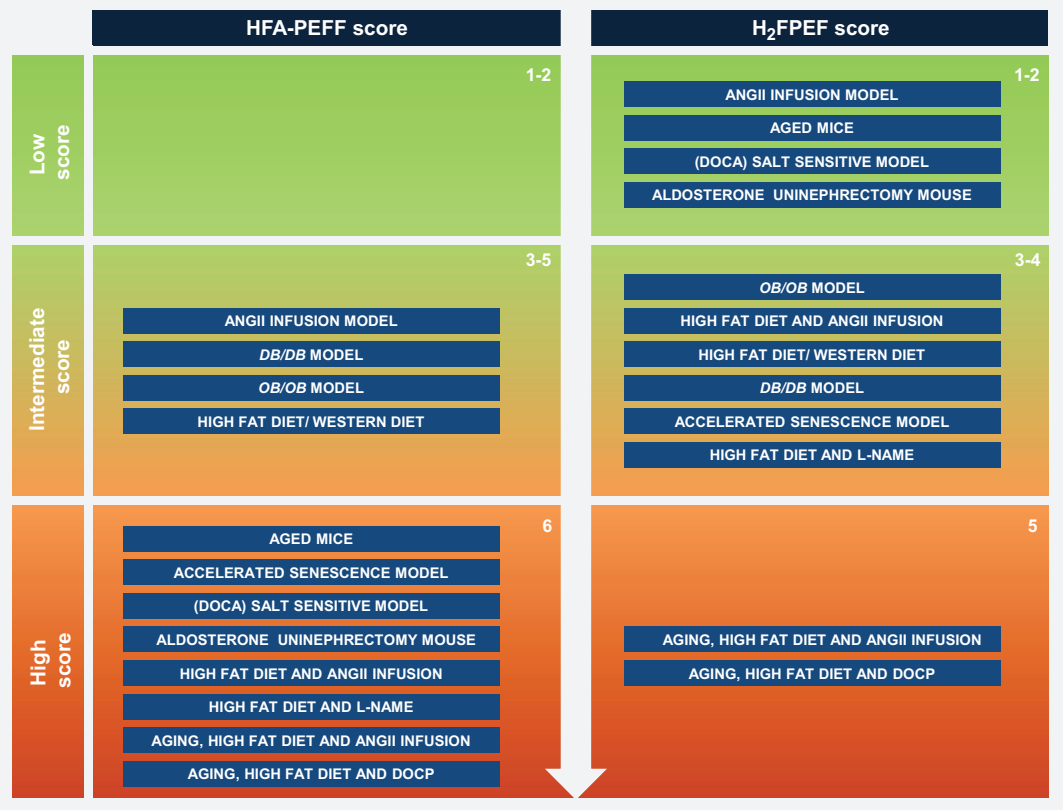


Figure 2 HFA-PEFF and H_2FPEF scores obtained by HF models. All HF models have been scored for cardiac and extra-cardiac domains of HFA-PEFF and H_2FPEF scores. Based upon these scores, mouse HF models are differentiated into more or less likely to fulfil the criteria of the HFA-PEFF or H_2FPEF score. If we solely record the scores, several of so-called HFpEF models would have obtained high scores due to functional or morphological features, while signs of lung congestion or exercise impairment were absent and levels of natriuretic peptides low. ANGII, angiotensin II; DOCA, deoxycorticosterone acetate; DOCP, desoxycorticosterone pivalate; *db/db*, leptin receptor-deficient model; HFpEF, heart failure with preserved ejection fraction; L-NAME, *N*(ω)-nitro-L-arginine methyl ester; *ob/ob*, leptin-deficient model.

impression if signs and symptoms and clinical HFpEF features are present:

- *Increased natriuretic peptide levels.* Natriuretic peptide levels should be measured in plasma or LV tissue. Elevated natriuretic peptide levels play an important part in the HFA-PEFF score and also provide a global impression if HFpEF is likely to be present in animals.
- *Impaired exercise performance.* Impaired exercise capacity caused by skeletal muscle weakness, fatigue, or cardiovascular to muscle mismatch should be measured by voluntary or forced exercise. This is a typical feature of HFpEF, and analysis of exercise capacity, including assessment of skeletal muscle function, will provide essential information regarding HFpEF severity.[69–71]
- *Lung congestion.* Analysis of lung weight and pulmonary vasculature will be helpful to determine increased diastolic filling pressures and presence of diastolic dysfunction.

In case surrogate measurements of signs and symptoms and clinical HFpEF features (increased natriuretic peptides, preserved ejection fraction and increased comorbidity burden) are not present, the pre-clinical model does not meet the HFpEF criteria as suggested by the two scores and should therefore not be regarded as a pre-clinical HFpEF model.

A distinct cardiac phenotype with preserved systolic lv function with concentric hypertrophy and diastolic dysfunction

- *Assessment of systolic cardiac function.* Systolic cardiac function should be assessed by transthoracic echocardiography and should include measurement of LV dimensions to assess concentric hypertrophy and LV systolic function. Post-mortem analysis (weighing and staining) of the total heart and LV should take place to assess amount of cardiac hypertrophy and fibrosis.
- *Assessment of diastolic function.* Diastolic function should be determined by morphological criteria (atrial enlargement) or functional parameters. In mice, evaluation of diastolic function is complex and the E/A and *E*/*e*′ ratio is difficult to assess and highly variable.[72] Global longitudinal strain (GLS) and reverse peak longitudinal strain rate (RPLSR) are easily obtained, highly reproducible, and

Table 1 Validation of HFpEF mouse models by HFA-PEFF and H_2FPEF scores

Validation of HFpEF mouse models by HFA-PEFF and H_2FPEF scores

Model	Pretest assessment of signs and symptoms, clinical HFpEF features and biological factors (age and sex)									HFA-PEFF score							H_2FPEF score						
	Preserved EF	Sex	Age (months)	Lung congestion	Impaired Exercise capacity	Comorbidity burden				Functional aspects	Morphological aspects				Increased natriuretic peptides	Total points	Obesity	Hyper-tension	Atrial Fibrillation	Pulmonary hyper-tension	Age	Diastolic dys-function	Total points
						Hyper-tension	Obesity	T2DM	Renal Dys-function	Diastolic dysfunction	Left atrial enlarge-ment	Left ventricular mass	Increased wall thickness	Concentric hyper-trophy									
Low HFpEF likelihood																							
Aldosterone uninephrectomy mouse	Yes	M	3	Yes	Yes	Yes	Yes	Yes	N/A	Yes	N/A	Yes	Yes	Yes	Yes	**6**	No	Yes	No	N/A	No	Yes	**2**
High fat diet/ Western diet	Yes	M/F	3–16	Yes	Yes	Yes	Yes	Yes	No	Yes	N/A	Yes	Yes	Yes	No	**4**	Yes	No	No	N/A	No	Yes	**4**
Aged mice (24–30 months)	Yes	M	24–30	Yes	Yes	No	No	No	No	Yes	N/A	Yes	Yes	Yes	Yes	**6**	No	No	No	N/A	Yes	Yes	**2**
Angiotensin-II infusion models	Yes	M/F	3	Yes	Yes	Yes	No	No	No	Yes	N/A	Yes	Yes	Yes	Yes	**6**	No	Yes	No	yes	No	No	**2**
Accelerated senescence model (SAMP)	Yes	F	3–12	No	Yes	Yes	No	No	N/A	Yes	Yes	Yes	Yes	Yes	Yes	**6**	No	Yes	No	N/A	Yes	Yes	**4**
Leptin receptor-deficient model (*db/db*)	Yes	M/F	3	No	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	**4**	Yes	Yes	No	N/A	No	Yes	**4**
Leptin-deficient model (*ob/ob*)	Yes	M/F	3	No	Yes	No	Yes	Yes	No	Yes	No	Yes	Yes	Yes	No	**4**	Yes	Yes	No	Yes	No	Yes	**3**
(DOCA) salt-sensitive model	Yes	M	3	No	No	Yes	No	No	No	Yes	N/A	Yes	Yes	Yes	Yes	**6**	No	No	No	No	No	Yes	**1**
High fat diet and angiotensin II	Yes	M	3	No	No	Yes	Yes	Yes	N/A	Yes	N/A	Yes	Yes	Yes	Yes	**6**	Yes	Yes	No	N/A	No	Yes	**3**
High HFpEF likelihood																							
High fat diet and L-NAME	Yes	M/F	3	Yes	Yes	Yes	Yes	Yes	N/A	Yes	N/A	Yes	Yes	Yes	Yes	**6**	Yes	Yes	No	N/A	No	Yes	**4**
Aging, high fat diet and angiotensin II	Yes	F	22	Yes	Yes	Yes	Yes	Yes	N/A	Yes	Yes	Yes	Yes	Yes	Yes	**6**	Yes	Yes	No	N/A	Yes	Yes	**5**
Aging, high fat and DOCP	Yes	M/F	18	Yes	Yes	Yes	Yes	Yes	N/A	Yes	N/A	Yes	Yes	Yes	Yes	**6**	Yes	Yes	No	N/A	Yes	Yes	**5**

HF models are scored for signs and symptoms or clinical HFpEF features, included age, sex, as well as cardiac and extra-cardiac domains of HFA-PEFF and H_2FPEF scores. Based upon these scores, mouse HF models were differentiated into more or less likely to fulfil the criteria of the human HFpEF situation, with higher scores representing pre-clinical HF models that most resembled clinical HFpEF. Models that presented full signs and symptoms and clinical HFpEF features are shown in the high HFpEF likelihood box.

db/db, leptin receptor-deficient model; DOCA, deoxycorticosterone acetate; DOCP, desoxycorticosterone pivalate; EF, Ejection fraction; L-NAME, *N*(ω)-nitro-L-arginine methyl ester; *ob/ob*, leptin-deficient model; T2DM, type 2 diabetes mellitus.

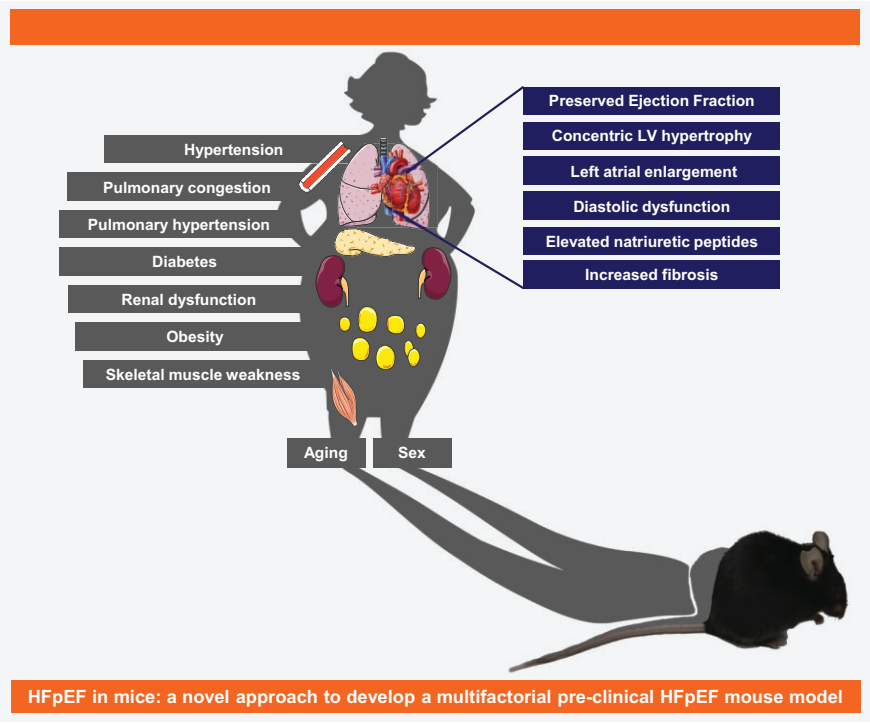


Figure 3 HFpEF in mice: a novel approach to develop a multifactorial pre-clinical HFpEF mouse model. The following clinical HFpEF features are essential to develop a reliable and accurate pre-clinical HFpEF model: (1) pulmonary congestion and elevated natriuretic peptides; (2) a distinct cardiac phenotype with preserved systolic LV function with concentric hypertrophy, fibrosis, atrial enlargement and diastolic dysfunction; (3) extra-cardiac comorbidities such as hypertension, obesity, T2DM and renal dysfunction and skeletal muscle weakness; and (4) incorporate and evaluate the effect of sex and aging. LV, left ventricle. Parts of the figure were drawn by using pictures from Smart Servier Medical Art (http://smart.servier.com), licensed under a Creative Commons Attribution 3.0 Generic License (https://creativecommons.org/licenses/by/3.0/).

have therefore to be integrated as indices of diastolic dysfunction in mice.[73,74] Post-mortem analysis (weighing) of atria should take place to evaluate atrial enlargement.

- *Assessment of cardiac hemodynamics*. Although considered as gold standard for diagnosis of HFpEF, invasive hemodynamic measurements are performed to a limited scale in humans due to a lack of expertise, availability, risks, and costs. A distinct advantage in animal models is that this gold standard assessment can be done more easily and more frequently but requires experience to be reliable. Invasive hemodynamic measurements provide information on intracardiac volumes, filling pressures, contractile and relaxation forces and derivate measures such as tau, d*P*/d*T* of the LV. Although measurements of systolic pulmonary artery pressure and pulmonary capillary wedge pressure yield additional information about diastolic function and pulmonary hypertension, measuring right-sided invasive hemodynamics presents more of a challenge in pre-clinical models and may not be required if gold standard left-sided invasive hemodynamics are already evaluated.

Extra-cardiac comorbidities such as hypertension, obesity, type 2 diabetes mellitus, and renal dysfunction

Assessment of extra-cardiac features of HFpEF should take place in all pre-clinical HFpEF models. This assessment should include evaluation of several comorbidities that are closely related to the development of HFpEF.

- *Hypertension*. Assessment of hypertension can be performed in several ways, including invasive hemodynamic measurements at sacrifice or by using tail-cuff measurements or continuous registrations throughout the study period.
- *Renal function*. Plasma should be obtained to determine kidney function. Post-mortem analysis of kidneys should take place (weighing + staining).
- *Obesity*. Mice should be repeatedly weighed during the experiment. Body mass composition should be determined throughout the experiment and prior to sacrifice.
- *T2DM*. Fasting plasma glucose levels or glycated hemoglobin should be obtained throughout the experiment. Glucose tolerance can be evaluated by oral glucose tolerance test and insulin sensitivity can be tested by insulin tolerance test.
- *Skeletal muscle weakness*. Post-mortem analysis of skeletal muscle should take place to evaluate reduced mass, and address impaired skeletal oxidative metabolism and abnormal skeletal muscle composition.

AF is a well-known comorbidity for HFpEF and represents an important part of the H_2FPEF score (three points if AF is present). Unfortunately, induction of AF in mice is challenging and so far none

of the experimental AF models resemble typical clinical HFpEF characteristics.[75–77] We therefore excluded AF from this section.

Effect of sex and aging

Epidemiological evidence suggests that HFpEF is highly represented in older women.[78] The effect of aging and sex should therefore be taken into account when developing a pre-clinical model.

- *Aging*. The life span of a rodent is shorter than humans, and mice are already considered 'old' after 18 months and 'very old' when >24 months.[79] Aging may represent an important contributing factor to the development of HFpEF and should therefore be considered when studying HFpEF.[57,80]
- *Female sex*. Sex-specific differences are known to exist in humans and mice [4,12,81–86] and for various interventions, young female mice have been shown to be less susceptible to develop a cardiac phenotype as compared to young males.[87,88] Hormonal differences or hormonal changes (such as menopause) are thought to play an important role in the increased cardiovascular risk profile of older females.[21,89] Interestingly, the development of LV hypertrophy may also occur in a sex-specific manner: females more often display concentric remodeling[89] while males develop eccentric LV remodeling.[90] Since the meaning of these differences are not fully understood yet we strongly advise to develop pre-clinical HFpEF models that take into account the effect sex may have. At the very least, investigators may consider including females rather than performing exclusively male experiments as is often the case.

Validation and translation of the H2FPEF and HFA-PEFF scores in animal models

For most experimental HFpEF models, mice are preferred small animals since they are easy to handle, quick to breed, allow genetic experiments, and are known to produce reliable and highly reproducible outcomes. Larger animal models of HFpEF, such as rat,[91–108] dogs[109,110] and pigs,[111–117] also exist (summarized in Supplementary material online, *Table S1*); nevertheless, ethical issues, difficulty in introducing high throughput genetic and molecular studies, cost, and duration of study limit large animal models. We included mice models that were widely used in HF research, and are presented as 'HFpEF' models, or were used to evaluate several HFpEF treatment options in the pre-clinical phase, often without translational success.

All models were scored for pre-clinical sign and symptoms or clinical HFpEF features (including age and sex), as well as cardiac and extra-cardiac domains of HFA-PEFF and H_2FPEF scores (*Table 1*). Based upon these scores, mouse HF models have been differentiated into more or less likely to fulfil the criteria of the HFA-PEFF or H_2FPEF score, schematically presented in *Figure 2*. In the *Graphical abstract*, we presented the models in less or high likelihood for HFpEF, including whether models with higher scores also present pre-clinical signs and symptoms or clinical HFpEF characteristics.

Angiotensin-II infusion models

Chronic stimulation of the ANGII type 1 receptor with ANGII infusion by osmotic mini-pumps is a well-known and reliable model to induce HF with cardiac hypertrophy and increased remodelling. Remodeling takes place with[118–122] or without[123] hypertension, depending on the dosage of ANGII. The ANGII effects seems to be strain specific: treatment with ANGII in Balb/c[124] mice typically results in lung congestion and LV dilatation, whereas treatment with ANGII in C57BL6 mice results in lung congestion, as well as exercise intolerance, concentric remodelling with fibrosis, and increased levels of natriuretic peptides.[120,122] ANGII treated mice develop diastolic dysfunction that includes worsening LV isovolumetric relaxation time, increased LV end-diastolic pressure and increased E/e'.[50,120–124] In mice, exogenous ANGII administration does not interfere with kidney function,[123] but may induce skeletal muscle alterations.[125] ANGII models, and especially the ANGII induced hypertension models, resemble cardiac features of human HFpEF to a large extent. Effects of age and obesity, however, are neglected in this model resulting in the following scores:

- Pretest assessment of signs and symptoms and clinical HFpEF features: lung congestion, hypertension and reduced exercise capacity.
- Total HFA-PEFF score: 6 (diastolic dysfunction, LV hypertrophy, increased natriuretic peptide levels);
- Total H_2FPEF score: 2 (hypertension and increased filling pressures).

Leptin receptor-deficient model (*db/db*)

Genetically modified *db/db* mice have a point mutation in the gene encoding for the leptin receptor that leads to malfunctioning of this receptor.[126] These mice are typically used for cardiometabolic research, especially for studies in the field of non-insulin dependent T2DM. Young *db/db* mice develop obesity, hyperglycaemia and severe dyslipidemia without hypertension.[127] The onset of symptoms in mice is severe and early in life, and therefore not directly translatable to the human situation in which progression of obesity and T2DM is a slower and chronic process. *db/db* mice have been from different strains, different ages and different sex[128] and results from studies performed in these mice are therefore not always comparable.

In general, *db/db* mice develop diastolic dysfunction including atrial enlargement, concentric hypertrophy, and fibrosis at older ages.[129,130] LV ejection fraction remains preserved, with decreased GLS rates after 16 weeks. Hypertension may be present, with[131,132] or without[133,134] ANGII infusion. Development of cardiac hypertrophy may already be present at early age (8–9 weeks[133,135]) or develops at a later point in time (up to 16 weeks[136,137]). Most *db/db* mice develop concentric hypertrophy, although eccentric hypertrophy has been observed as well.[138] Signs of congestion are usually not present in these mice, and natriuretic peptide levels are not elevated.[139,140]

- Pretest assessment of signs and symptoms and clinical HFpEF features: increased comorbidity burden (obesity and diabetes) and reduced exercise capacity.
- Total HFA-PEFF score: 4 (diastolic dysfunction, LV hypertrophy);
- Total H_2FPEF score: 4 (obesity, hypertension, diastolic dysfunction).

Leptin-deficient model (*ob/ob*)

The *ob/ob* is a leptin-deficient mouse that spontaneously develops obesity (within 4 weeks) and T2DM secondary to hyperglycaemia and hyperinsulinemia.[141,142] The mice develop concentric hypertrophy with diastolic dysfunction possible due to lipid accumulation.[143] The ejection fraction is preserved without congestion or exercise impairment and natriuretic peptide levels are unchanged or reduced.[144–146] The observed maladaptive cardiac alterations appear to be related to the loss of leptin mediated signaling and are reversed by recombinant leptin treatment.[129,147] However, obese HFpEF patients with leptin deficiency are rarely observed, so the *ob/ob* mice do not mimic the human HFpEF phenotype.[148]

- Pretest of signs and symptoms and clinical HFpEF features: increased comorbidity burden (obesity and diabetes) and reduced exercise capacity.
- Total HFA-PEFF score: 4 (diastolic dysfunction, LV hypertrophy);
- Total H_2FPEF score: 3 (obesity, diastolic dysfunction).

High fat diet/western diet

Obesity is an important comorbidity in patients with HFpEF and has been suggested to play an import role in (development of) HFpEF.[149,150] In pre-clinical models, unhealthy food consumption is mimicked by a high fat diet (HFD) (>60% fat of daily caloric intake) or by a Western diet (36% fat and 36% sucrose of daily intake). Both of these diets are able to induce an unfavourable cardiometabolic phenotype with obesity and glucose intolerance in young male and female animals[138,151–155] albeit in a strain-specific manner.[156–159] In older animals, the HFD appears to result in more profound cardiometabolic changes including hyperglycaemia and insulin resistance and more profound inflammation.[160,161] There may also be sex-specific effect as female mice tend to gain more weight than age-matched male littermates.[81,138,154–164]

Besides an unfavourable cardiometabolic phenotype, these models result in concentric LV hypertrophy with preserved ejection fraction, and mild to moderate diastolic dysfunction.[154,163,164] Furthermore, pulmonary hypertension has been described as well as increased levels of cardiac fibrosis.[164,165] Pulmonary congestion is absent and levels of natriuretic peptides are usually not elevated.[166] Renal dysfunction may occur after long term diet (>20 weeks) in young mice or at earlier point in time in aged mice.[161,167,168] Mice fed on an HFD or Western diet typically show reduced exercise capacity, most likely related to their obese state as skeletal muscle weakness is not observed in these mice.[71,157,169]

- Pretest assessment of signs and symptoms and clinical HFpEF features: increased comorbidity burden (obesity and pre-diabetes) and reduced exercise capacity.
- Total HFA-PEFF score: 4 (diastolic dysfunction, LV hypertrophy).
- Total H2FPEF score: 4 (obesity, pulmonary hypertension, diastolic dysfunction)

Aged mice (24–30 months)

Similar to humans, natural aging in mice (with or without dietary intervention) is a main driver of development of a maladaptive cardiac HFpEF phenotype.[170] At an age of 24–30 months, mice recapitulate many hallmarks of human HFpEF pathophysiology, including diastolic dysfunction, concentric hypertrophy with fibrosis and reduced exercise capacity.[171,172] This mice furthermore have lung congestion and increased natriuretic peptide levels. Hypertension or T2DM, however, have not been described.

- Pretest assessment of signs and symptoms and clinical HFpEF features: lung congestion, increased natriuretic peptide levels, reduced exercise capacity, but no comorbidity burden.
- Total HFA-PEFF score: 6 (diastolic dysfunction, LV hypertrophy);
- Total H2FPEF score: 2 (age, diastolic dysfunction).

Accelerated senescence model (SAMP)

Senescence accelerated prone (SAMP) mice belong to a strain of mice that were generated by selective inbreeding of AKR/J mice.[173] These mice show accelerated senescence and age-related pathological phenotypes, similar to aging disorders seen in humans. In addition, they start displaying features of aging at younger age (10 months) than normal mice (8 months).[174] Deleterious mutations in the DNA repair genes are to be involved in their genetic vulnerability for enhanced aging, and specific gene analyses show involvement of oxidative and stress response pathways.[175] SAMP mice develop age-related diastolic dysfunction with atrial enlargement and adverse cardiac remodelling including LV hypertrophy and fibrosis.[102,159,176] Levels of natriuretic peptides are elevated in these mice.[159] When fed a Western diet, SAMP mice also develop hypertension and lung congestion, albeit without obesity or T2DM.[159] It has not been described if female or male SAMPs age differently.

- Pretest assessment of signs and symptoms and clinical HFpEF features: increased natriuretic peptide levels, lung congestion and reduced exercise capacity.
- Total HFA-PEFF score: 6 (diastolic dysfunction, LV hypertrophy, elevated natriuretic peptides).
- Total H_2FPEF score: 4 (hypertension, effect of aging, increased filling pressures).

Progress in pre-clinical HF models: development of multifactorial models

The abovementioned models are mostly unifactorial disease models that use one perturbation to induce HF. More recently, progress has been made in the development of pre-clinical HFpEF models and this has led to multifactorial models that use two or more perturbations to mimic the human HFpEF phenotype. In the following section, we will again use the HFA-PEFF and H_2FPEF score to describe and validate a traditional multifactorial model as well as newer multifactorial HFpEF models.

Deoxycorticosterone acetate salt-sensitive model

The deoxycorticosterone acetate salt-sensitive model was already developed in 1969 to study hypertension in young mice and rats.[177] This model relies upon a combination of multiple perturbations including administration of deoxycorticosterone acetate, increased salt intake (addition of 1% NaCl to drinking water) and

uninephrectomy. This typically results in cardiac hypertrophy with fibrosis, increased levels of natriuretic peptides, while blood pressure remains unchanged or only mildly increased.[178,179] LV function remains preserved while moderate diastolic dysfunction can be observed.[180] Nevertheless, these mice do not display lung congestion.[181] Again, the effect of age and sex has not been described in this model.

- Pretest assessment of signs and symptoms and clinical HFpEF features: increased natriuretic peptide levels, and reduced exercise capacity.
- Total HFA-PEFF score: 6 (diastolic dysfunction, LV hypertrophy, increased levels of natriuretic peptides);
- Total H_2FPEF score: 1 (diastolic dysfunction).

Aldosterone uninephrectomy mouse

Impaired renal function is frequently observed in patients with HFpEF. Renal dysfunction may be attributed to fluid overload, blood pressure elevation, and thus congestion.[182] In C57BL6 or FB/N background, the combination of uninephrectomy and aldosterone infusion results in the development of hypertension, lung congestion, and reduced exercise capacity without obesity or T2DM.[183,184] Preserved LV ejection fraction is observed with concentric remodelling, mild-to-moderate diastolic dysfunction, and increased levels of natriuretic peptides.[185–188] The effect of female sex or aging is unknown and obesity or T2DM is not observed.

- Pretest assessment of signs and symptoms and clinical HFpEF features: lung congestion, increased natriuretic peptide levels and reduced exercise capacity.
- Total HFA-PEFF score: 6 (diastolic dysfunction, LV hypertrophy, increased levels of natriuretic peptides);
- Total H_2FPEF score: 2 (hypertension, increased filling pressures).

Combinatory model of high fat diet and L-NAME

Schiattarella *et al.*[169] were the first to present a two-hit pre-clinical mouse model that resembles human HFpEF. In short, C57BL/6N wild-type mice were subjected to a combination of HFD and hypertension that was induced by L-NAME (constitutive nitric oxide synthase inhibitor). They observed that mice that were subjected to both stress factors developed a typical HFpEF phenotype, including lung congestion and reduced exercise tolerance and increased natriuretic peptides. On the contrary, mice that were only exposed to one stressor did not develop this phenotype.[169] More recently, sex-dependent effects have also been shown: young female mice were more resilient for development of HFpEF, as the combination of high-fat and L-NAME resulted in a more attenuated cardiac phenotype as compared to young male mice.[189] The effect of aging was not studied.

- Pretest assessment of signs and symptoms and clinical HFpEF features: increased natriuretic peptides, lung congestion, reduced exercise capacity, and increased comorbidity burden (hypertension, obesity and pre-diabetes).
- Total HFA-PEFF score: 6 points (increased natriuretic peptides, diastolic dysfunction, concentric LV hypertrophy).
- Total H_2FPEF score: 4 (obesity, hypertension, increased filling pressures).

Combinatory model of high fat diet and ANGII infusion

The combination of HFD and ANGII infusion induces hypertension, obesity and T2DM in young male mice.[151,190,191] This intervention also results in preserved LV function with diastolic dysfunction, concentric hypertrophy with fibrosis and increased natriuretic peptides. However, signs and symptoms or clinical features of HFpEF, if any, appear to be very mild since lung congestion in young animals is absent and effect on exercise capacity is unknown.[151,190–192]

- Pretest assessment of signs and symptoms and clinical HFpEF features: increased natriuretic peptide levels, increased comorbidity burden (hypertension and pre-diabetes);
- Total HFA-PEFF score: 6 (diastolic dysfunction, LV hypertrophy, elevated levels of natriuretic peptides);
- Total H_2FPEF score: 4 (obesity, hypertension, increased filling pressures).

Combinatory model of aging, high fat diet, and ANGII infusion

We have recently developed a multifactorial mouse model that combines aging (18–22 months) with HFD and ANGII infusion.[193] In these older female C57BL6/J mice, a HFpEF-like phenotype is present including concentric LV hypertrophy and LV fibrosis, diastolic dysfunction, lung congestion, increased natriuretic peptide levels, and elevated blood pressures. The effect of sex has not been studied yet.

- Pretest assessment of signs and symptoms and clinical HFpEF features: lung congestion, increased natriuretic peptide levels, reduced exercise capacity, and increased comorbidity burden (hypertension, obesity and pre-diabetes);
- Total HFA-PEFF score: 6 (diastolic dysfunction, concentric LV hypertrophy, elevated natriuretic peptide levels);
- Total H_2FPEF score: 5 (obesity, hypertension, elderly, increased filling pressures).

Combinatory model of aging, high fat diet and desoxycorticosterone pivalate

A very recent study by Deng *et al.*[194] used a combinatory model of 16 months of ageing, long-term HFD (13 months) and 3 months of desoxycorticosterone pivalate challenge in mice to induce a HFpEF-like phenotype. Their model resulted in many typical HFpEF features, including lung congestion, hypertension and impaired exercise tolerance. They also showed diastolic dysfunction, LV hypertrophy, fibrosis and increased levels of natriuretic peptides. Both sexes were included but not further studied.

- Pretest assessment of signs and symptoms and clinical HFpEF features: lung congestion, increased natriuretic peptide levels, reduced exercise capacity and increased comorbidity burden (hypertension, obesity and pre-diabetes);
- Total HFA-PEFF score: 6 (diastolic dysfunction, LV hypertrophy, elevated natriuretic peptide levels);
- Total H_2FPEF score: 5 (obesity, hypertension, elderly, increased filling pressures).

Conclusion

HFpEF remains a major public health problem worldwide with still increasing prevalence and incidence. So far, HFpEF treatment mostly focuses on symptom reduction since HFpEF-specific drugs do not exist. Despite numerous efforts to develop HFpEF-specific drugs, bench-to-bedside translation has not been successful, and this may, at least partly, be due to the lack of pre-clinical HFpEF models that adequately recapitulate the complexities of the human condition.

HFpEF is a multifactorial disease in which comorbidities contribute to the pathophysiology of the clinical syndrome. While this complicates the development of preclinical models, progress in the field will be aided by consensus on key elements that a HFpEF animal model should manifest. The recent development of two clinical HFpEF scores has led to a novel clinical standard for defining the key clinical features of HFpEF. This state-of-the-art review is the first to apply clinical scores to HFpEF mouse models to improve putative applicability and translational value of pre-clinical HFpEF research. It proposes a novel approach to follow when performing a pre-clinical HFpEF study to optimize bench-to-bed translation and provide a checklist for small HFpEF animal models. Although this checklist may not capture all human HFpEF variables, it will help to provide better and more relevant small animal HFpEF models with better putative application and translational value. So far, most of the pre-clinical models do not fully meet these criteria (presented in *Graphical abstract*). Of course, pathophysiology of the mouse heart cannot be translated to humans 1 on 1, and translation of pre-clinical findings to human conditions should always be done cautiously. Of note, clinical studies should be challenged as well to account for diverse HFpEF physiology to optimize bench-to-bed translation.

This review furthermore describes some multifactorial models that resemble human HFpEF to a large extent, and suggests that these small animal models remain attractive models for future HFpEF research. Based on this review, we advocate that future HFpEF pre-clinical studies that test potential new therapeutic agents should consider use of multiple HFpEF animal models so that their effects can be tested on multiple HFpEF phenotypes. Following this approach we believe that pre-clinical HFpEF models will be able to help fill major gaps in HFpEF pathophysiology and will eventually facilitate development of novel HFpEF therapeutics.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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