Supplementary Material

Supplementary Table 1: Difference of HFpEF between Animal models and Human studies

Criteria	Animal Models	Human Studies	Ref
HFpEF Pathology	Animal models often fail to fully replicate the complexity of human HFpEF, particularly in terms of the diverse comorbidities and multi-organ involvement seen in humans. Many models exhibit limited progression to the advanced stages of HFpEF, especially in the context of chronic inflammation and fibrosis.	Human studies directly reflect the pathological complexity of HFpEF, encompassing key features like diastolic dysfunction, systemic inflammation, endothelial dysfunction, and myocardial fibrosis. Human studies also enable observation of HFpEF progression over time, making them critical for evaluating disease pathophysiology.	[1,2]
Metabolism	Animal models often exhibit different metabolic profiles, including varying fat distribution and insulin sensitivity, which	Human studies offer more accurate insight into how metabolic diseases like obesity, insulin resistance, and metabolic syndrome contribute to	[2,3]

	complicates the extrapolation of findings to human metabolic responses. Moreover, animals may not fully replicate human responses to obesity and type 2 diabetes mellitus, which are significant risk factors in HFpEF.	HFpEF. Metabolic changes, such as visceral fat accumulation and insulin resistance, have been shown to exacerbate inflammation and fibrosis in HFpEF, thus impacting both disease progression and treatment efficacy.	
Molecular Studies	Animal studies enable detailed exploration of cellular processes, such as autophagy and ER stress, at a molecular level. They allow for controlled manipulation of variables to assess the effects of these mechanisms on cardiac function and remodelling. However, animal models often fail to completely reflect the human-specific molecular interactions.	Human studies often focus on validating biomarkers and the molecular mechanisms identified in animal models. Recent advancements include the identification of autophagic markers like LC3 and Beclin-1, and stress markers like GRP78 and CHOP in human myocardial tissue, helping link molecular dysregulation with clinical disease severity.	[4,5]

Biomarkers	Animal models enable identification of biomarkers under controlled conditions, including autophagic and ER stress markers like LC3, Beclin-1, and GRP78, that can be linked to cardiac dysfunction and remodelling. However, the relevance to human pathology is often less clear due to differences in biomarker regulation.	Human studies provide a direct validation of biomarkers such as GRP78, CHOP, and p62, which have been shown to correlate with HFpEF severity and myocardial fibrosis. These biomarkers are critical in both diagnosis and monitoring disease progression in clinical settings. Their utility in detecting early myocardial dysfunction in HFpEF is currently under investigation.	6–8
Treatment Effectiveness	Animal models often provide preliminary data on the effectiveness of new pharmacological interventions, such as autophagy modulators or ER stress inhibitors. However, differences in pharmacokinetics and pharmacodynamics complicate the direct translation of these	Clinical studies provide more robust evidence for the effectiveness of therapies, such as renin–angiotensin–aldosterone system (RAAS) inhibitors, spironolactone, and new drugs like sacubitril/valsartan. These therapies aim to modify disease progression by targeting neurohumoral mechanisms, fibrosis, and	9,10

	findings to humans. Furthermore, results may be less reliable due to species-specific responses to drugs.	systemic inflammation. Clinical trials focusing on autophagy and ER stress modulation are in the early stages, with promising results for improving outcomes in HFpEF.	
Hormonal Balance	Animal studies on ovariectomised female rodents, which mimic postmenopausal oestrogen deficiency, has demonstrated increased oxidative stress, mitochondrial dysfunction, and impaired NO signalling, leading to left ventricular remodelling and diastolic dysfunction.	In humans, hormonal balance, particularly oestrogen, is crucial in the pathophysiology of HFpEF, contributing to gender differences in disease frequency and severity. In premenopausal women, oestrogen reduces oxidative stress and mitochondrial ROS generation, but in postmenopausal women, oestrogen deprivation increases oxidative	[11,12]

	damage and reduces NO production.[10] Recent research suggests that oestrogen therapy could have therapeutic benefits for women with HFpEF.

Supplementary Table 2. Clinical Utility of Autophagy and ER Stress Biomarkers in HFpEF

Biomarker	Clinical role in HFpEF	Advantages	Challenges / Limitations	Ref
LC3 (LC3-I/LC3-II)	Autophagosome marker; LC3-II reflects autophagic flux. Increased expression in diabetic myocardium; proposed in autophagic cardiomyopathy	Widely used; robust antibodies; captures flux when paired with p62	Tissue/context dependent; LC3-II may accumulate with blocked flux; circulating assays not standardised	[4,13]
Beclin-1	Initiates autophagosome nucleation; upregulated in T2D myocardium, linking metabolic stress to HFpEF remodelling	Central node of autophagy initiation; mechanistically informative	Non-specific; influenced by upstream signals; no validated clinical cut-offs	[4]
p62 / SQSTM1	Selective autophagy adaptor; accumulates when	Directionality (build-up suggests impaired flux); mechanistic link to proteostasis	Affected by proteasome inhibition and general	[14,15]

	autophagy impaired; used with LC3 in cardiomyopathy		stress; temporal variability	
ATG7	E1-like enzyme essential for autophagosome membrane expansion and LC3 lipidation; supports cardiometabolic homeostasis. ATG7 activation reverses doxorubicin cardiotoxicity via restoration of autophagy, and ATG7 loss in cardiomyocytes promotes senescence and cardiac dysfunction	Proximal readout of autophagic capacity; strong mechanistic/therapeutic relevance demonstrated in cardiac models	No routine clinical assay; evidence largely preclinical/early translational; tissue assays required; pathway effects can be context-dependent	16

GRP78 / BiP	ER stress chaperone; elevated in HFpEF tissue/circulation; correlates with severity and prognosis	Measurable in blood; strong ER stress signal; reflects adaptive UPR	Upregulated in many diseases (cancer, metabolic); specificity limited	[7,17]
CHOP (DDIT3)	Pro-apoptotic ER stress effector; elevated in HFpEF and linked with remodelling/apoptosis	Tracks maladaptive ER stress; detectable by immunohistochemistry (IHC)/enzyme-linked immunosorbent assay (ELISA)/qPCR	Not heart-specific; induced by various stresses; timing-dependent	[11]
ATF6	UPR sensor/effector; nuclear ATF6 upregulated in HF myocardium	Direct readout of UPR arm; mechanistically specific	Primarily tissue-based; dynamic; limited assays	[18–20]

IRE1α (± XBP1s)	UPR sensor mediating XBP1 splicing; overactivation linked to fibrosis/inflammation; Pak2 protective	Functional pathway marker; therapeutic relevance	Assays complex; tissue variability; thresholds lacking	[21,22]
PERK–eIF2α	UPR arm reducing protein translation; intersects with autophagy–apoptosis in cardiac stress	Phospho-specific antibodies available; mechanistically informative	Phospho-epitopes labile; timing critical; mainly research	[18,23]
Caspase-3	Executioner caspase; elevated with GRP78 in HFpEF cohorts; associated with prognosis	Widely available assays; integrates apoptosis pathway	Non-specific to ER stress/myocardium; transient activation may be missed	[11]

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