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POSITION PAPER

An integrative translational approach to study heart failure with preserved ejection fraction: a position paper from the Working Group on Myocardial Function of the European Society of Cardiology

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As heart failure with preserved ejection fraction (HFpEF) rises to epidemic proportions, major steps in patient management and therapeutic development are badly needed. With the current position paper we seek to update our view on HFpEF as a highly complex systemic syndrome, from risk factors and mechanisms to long-term clinical manifestations. We will revise recent advances in animal model development, experimental set-ups and basic and translational science approaches to HFpEF research, highlighting their drawbacks and advantages. Directions are provided for proper model selection as well as for integrative functional evaluation from the *in vivo* setting to *in vitro* cell function testing. Additionally, we address new research challenges that require integration of higher-order inter-organ and inter-cell communication to achieve a full systems biology perspective of HFpEF.

Keywords Heart failure with preserved ejection fraction • Animal models • Experimental evaluation • Molecular biology • Myocardial function

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Introduction

After a long period of omission, past years have been prolific in trials addressing heart failure with preserved ejection fraction (HFpEF). Epidemiological trends suggest that with increased longevity and escalating co-morbidity burden, the prevalence of HFpEF may rise to epidemic proportions. Mortality ranges from 30 to 60% at 5 years, hospitalization rate is high and quality of life (QOL) is severely impaired. Several randomized clinical trials (RCTs) have attempted to halt disease progression and mitigate morbidity and mortality targeting various potential pathophysiological mechanisms, all with inconclusive or neutral results. A detailed overview of these RCTs is out of the scope of the current work and can be found elsewhere.[1,2] Interestingly, changes in lifestyle such as exercise training and caloric restriction have shown the most promising results in short-term trials,[3,4] raising the possibility that pleiotropic effects will be needed to change the course of HFpEF.[5] Lack of progress in pharmacological patient management warrants not only for better designed trials with well defined enrolment criteria and end-points, but also for a clearer understanding of pathophysiology.[6–8] To date, translational and basic science were unable to support therapeutic development. Indeed, while a multitude of data from experimental models has been gathered from organ baths down to intracellular mechanisms involved in cardiac relaxation and compliance, vascular function, and inflammation, these detailed mechanical, biochemical and molecular insights derived from basic science are yet to be linked in a full extent to preclinical models and sophisticated patient phenotyping. This position paper focuses on current knowledge on the pathophysiology of HFpEF, available animal models and experimental methodologies. From these data, we propose directions for future research in the translational field.

Diagnosis of heart failure with preserved ejection fraction

Diastolic function is dictated by left ventricular (LV) relaxation and compliance which jointly enable filling at low pressure[9] (*Figure 1*). Disturbances in any of these lead to diastolic dysfunction (DD). Asymptomatic DD [by some denoted as preclinical heart failure (HF)] is common in the community. When carefully studied, patients often develop reduced QOL and show increased cardiovascular risk.[10] Importantly, follow-up studies revealed frequent progression to HFpEF.[11] Simplistically, the diagnosis of HFpEF relies on signs and symptoms of lung congestion that cannot be attributed to other causes, preserved or normal ejection fraction and markers of diastolic function impairment.[12] The gold standard to diagnose HFpEF would be invasive haemodynamic evaluation with exercise testing because it clearly documents cardiac failure development (by rising filling pressures and inability to increase cardiac output) during effort[13,14] but clearly non-invasive surrogates are warranted because invasive testing carries risk and is not feasible in every patient. The diagnosis of HFpEF remains disputed. The 2016 ESC guidelines require at least symptoms, objective signs of HF, and some degree of structural or functional deficit. The latter is usually measured by echocardiography, and may include LV hypertrophy (LVH), increased left atrial volume, and various abnormalities associated with DD.[15] Although DD has always been considered a key element, only two-thirds of patients show DD at rest in some RCTs.[16,17] This simple observation underscores that there is no solid consensus on the diagnosis of HFpEF. Indeed, although DD is a dominant feature of HFpEF, most experts now view it as a complex syndrome in which multiple cardiac, vascular and non-cardiac determinants come into play to impair cardiovascular reserve[18,19] (*Figure 2*). While in HF with reduced ejection fraction (HFrEF) a sudden insult leads to myocyte loss, functional impairment and self-amplifying neurohumoral cascades, HFpEF is a slowly progressive process without an index event. Ageing and co-morbidities progressively drive dysfunction by way of altered load conditions, inflammation and complex systemic changes. The direct effects of ageing explain the predominance of HFpEF in the elderly, who have long-term co-morbidities and impaired cardiovascular reserve. For practical reasons in basic research a single organ–single stressor approach is usually favoured. Indeed, ageing and multiple co-morbidities are difficult to mimic in the laboratory. Nevertheless, several new insights from experimental studies on ageing and co-morbidities have now emerged and could propel our knowledge of HFpEF. We will discuss them.

It should be highlighted that ejection fraction (EF) is load-dependent[9] and overestimated in hypertrophy due to increased myocardial thickening.[20] Load-independent indexes show impaired baseline contractility and poor response to exercise.[21,22] Regardless of its limitations, current guidelines[15] base their diagnostic criteria on EF. Follow-up reveals that patients classified under HFpEF or HFrEF would later fall in the opposite category,[23] echocardiography poorly tracks individual evolution,[24] and reliance on resting parameters is inadequate because HFpEF begins with effort intolerance. Guidelines poorly incorporate this concept, relying mostly on data acquired from inpatients with decompensated HF and unusually high natriuretic peptide levels. Not surprisingly, sensitivity is poor when applied to RCTs enrolling stable patients[16] or outpatients with dyspnoea.[25] Effort testing may improve sensitivity of current ESC HFpEF diagnostic guidelines at the expense of reduced specificity.[14]

Mechanisms and phenotypes of heart failure with preserved ejection fraction

Cellular mechanisms

Endomyocardial biopsies reveal more extensive cardiomyocyte hypertrophy and myofibrillar density in HFpEF compared with HFrEF but no difference in collagen volume fraction.[26] Indeed, in *in vitro* set-up, HFpEF patients' cardiomyocytes are stiffer and more Ca^{2+} sensitive.[26] The sarcomeric protein titin is a key determinant of cardiomyocyte stiffness, both by isoform shift favouring

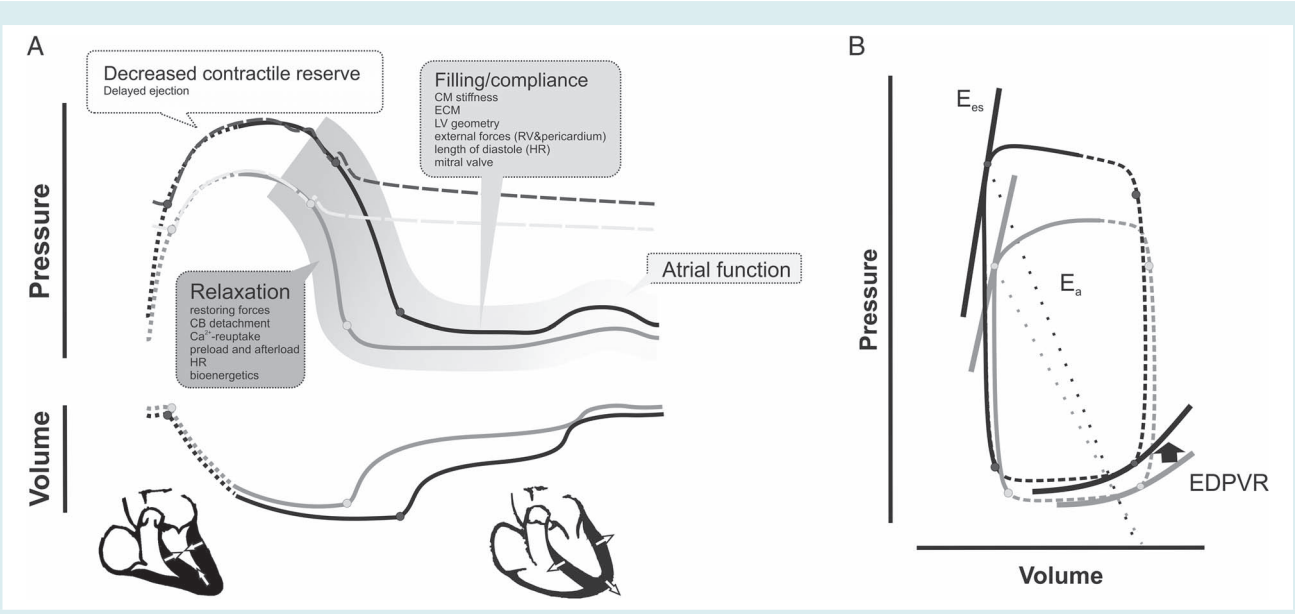

Figure 1 Cardiac mechanisms underlying diastolic dysfunction. Pressure and volume throughout the cardiac cycle are depicted as a function of time (*A*) and as pressure–volume (PV) loops (*B*). The main mechanisms governing left ventricular (LV) relaxation and filling as well as atrial contribution to LV filling are highlighted. Healthy (grey lines) and diastolic dysfunction tracings (black lines) are represented as dotted lines during active contraction and continuous lines from the beginning of active relaxation. The timings of valve opening and closure are denoted by circles. In (*A*) the corresponding aortic pressure tracings are also presented (light grey and grey dashed lines, respectively) whereas in (*B*) the end-systolic and end-diastolic PV relationships are shown. Notice that in (*A*) high afterload and reflected waves lead to delayed start and course of relaxation which along with lower compliance dictates slower filling at the expense of higher filling pressures and a proportionally larger dependence on atrial contraction. In (*B*) notice the upward shift in end-diastolic PV relationship (EDPVR) as well as impaired ventricular–vascular coupling due to the marked increase in effective arterial elastance (E_a) despite higher end-systolic elastance (E_{es}). CB, cross-bridge; CM, cardiomyocyte; ECM, extracellular matrix; HR, heart rate; RV, right ventricle.

the (stiff) N2B and by hypophosphorylation,[26] although hypophosphorylation seems to dominate. Other post-translational modifications may also contribute. Additionally, titin–actin interactions account for 40% of LV viscosity and thus play a major role in delayed relaxation.[27] Further, experimental data from an aortic banding model showed decreased Ca^{2+} transient amplitude and decay, which may contribute to impaired relaxation. Likewise, the ratio of sarcoendoplasmic reticulum Ca^{2+} ATPase to phospholamban content in HFpEF was decreased compared with the HFrEF patients' myocardia.[28] Various myofilamentary proteins may be modified post-translationally raising diastolic stress in HFpEF[29] as well as impaired bioenergetics (particularly high ADP) by way of actomyosin interactions.[30]

Interestingly, while fibrosis was held to be a major player, neither HFpEF patient biopsies nor animal models strongly support this hypothesis.[31] Indeed, HFpEF patients' biopsies demonstrate varying degrees of myocardial interstitial fibrosis[26] and autopsy studies reveal only minor increases in fibrosis.[32]

Ageing

Ageing lengthens relaxation and increases LV stiffness by collagen accumulation and cross-linking, cardiomyocyte loss, and reactive hypertrophy.[33] Neuroendocrine disturbances, mitochondrial dysfunction, increased oxidative stress, and fibroblast activation are well established ageing-associated pathways. Repeated heart beats rupture the elastin laminae of central vessels leading to dilatation and stiffening, loss of Windkessel effect and distal transmission of pulsatile pressure.[34] Increased wave velocity leads to earlier wave reflection. Although initial studies on hypertensive patients have shown that wall stress actually decreases in late systole, at the time of wave reflection,[35] population-based studies strongly suggest that wave reflection and augmented wall stress in late systole contribute to impaired relaxation.[36] Moreover, in patients referred for coronary angiography, wave reflection predicts worse cardiovascular outcomes, particularly when systolic function is preserved.[37] Indeed, augmented wall stress raises LV work, conversely decreasing diastolic perfusion pressure. Increased wall stress in turn raises end-systolic elastance and volume sensitivity while transmitted pulse pressure evokes endothelial dysfunction (ED). Increased arterial stiffening with exercise has been recently shown in HFpEF.[38] Measures of LVH, left atrial size, DD but also natriuretic peptides are age-dependent, which suggests that ageing represents to some extent physiological HFpEF. Clearly, ageing is an autonomous progressive process, so the boundaries between ageing and HFpEF remain uncertain. One of the main challenges, therefore, will be to provide a more uniform description of the ageing cardiac phenotype, in order to set it apart from HFpEF.

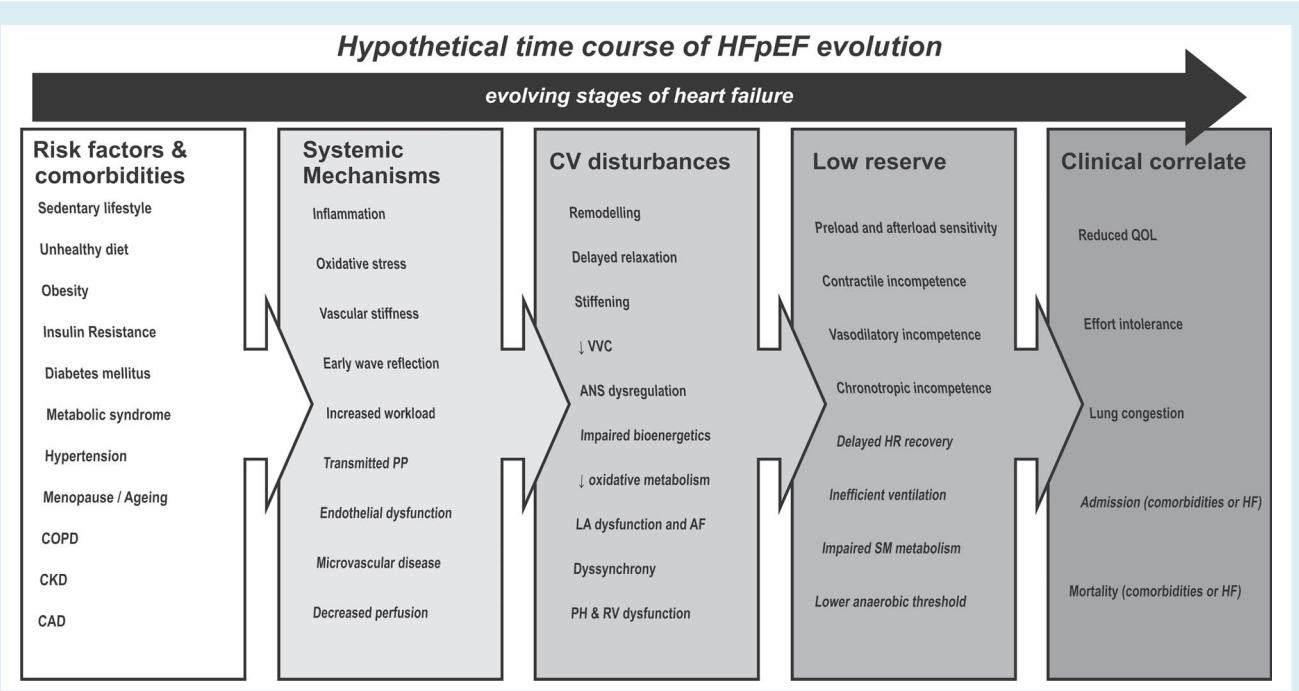

Figure 2 Time course of heart failure with preserved ejection fraction (HFpEF) evolution. The scheme highlights the long-term course and hypothetical progression from established risk factors and mechanisms to impaired cardiovascular (CV) function, decreased CV reserve and ultimately mortality. Timing of events and their order is of course variable between patients and still debatable, it is merely hypothetical. AF, atrial fibrillation; ANS, autonomic nervous system; CAD, coronary artery disease; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; HF, heart failure; HR, heart rate; LA, left atrial; PH, pulmonary hypertension; PP, pulse pressure; QOL, quality of life; RV, right ventricular; SM, skeletal muscle; VVC, ventricular–vascular coupling.

Gender

Although it is now realized that not only elderly females but also younger obese and diabetes mellitus (DM) men constitute HFpEF risk groups,[39] women tend to predominate in HFpEF cohorts. This finding may be explained because women more often reach an advanced age, but also by pathophysiological mechanisms. Gender differences in vascular biology and sex hormones may explain pre-menopausal preservation of elasticity and postmenopausal aortic stiffening. Aortic elasticity is lost after menopause, constituting a potential explanation for hypertrophic remodelling and HFpEF.[40] Loss of ovarian function also leads to ED and inflammation, which entail co-morbidities and HFpEF.[41]

Co-morbidities

It is well recognized that a higher burden of co-morbidities exists in HFpEF. The most prominent amongst co-morbidities are systemic arterial hypertension, obesity and DM, but a long list of other co-morbidities such as chronic obstructive pulmonary disease, renal dysfunction, sleep disordered breathing, hypothyroidism and anaemia have also been well documented.[42–44] Non-cardiac adverse events, other than typical HF end-point such as pump failure and sudden death are common[45] but it remains unclear whether they outweigh HF-related outcomes.[46] Competitive risk for non-cardiac death and events unrelated to the disease process itself poses a major hurdle to survival analysis in RCTs,[47] which may warrant a focus on secondary outcomes such as functional capacity. Conversely, co-morbidities themselves are an integral part of the HFpEF syndrome, and actively contribute to dysfunction and remodelling in HFpEF (*Figure 2*). Obesity and metabolic syndrome associate with DD well before DM, while DM may ultimately lead to cardiomyopathy.[48] To revise how each co-morbidity contributes to disease in the context of HFpEF is outside the scope of the current work and detailed overviews can be found elsewhere.[42] We must underscore, however, that HFpEF is not just the outcome of co-morbidities. Abnormalities in cardiovascular structure and function go beyond those explainable by co-morbidities alone. Co-morbidities, however, do influence phenotype and outcomes and should be aggressively managed.[39]

Microvascular and epicardial coronary artery disease

Recent progress in HFpEF research has suggested that HFpEF may in fact be a disease of the microvasculature. Studying the role of co-morbidities and inflammation created a new hypothesis based on coronary microvascular ED,[49] which was supported by findings at autopsy.[32] HFpEF patients show systemic microvascular dysfunction as well as coronary microvascular ED and rarefaction.[50] Interestingly, epicardial coronary artery disease (CAD) prevalence

was also higher at autopsy.[32] CAD is documented in many of HFpEF patients, pooled analysis of prospective HFpEF studies suggests that it is present in approximately 50% of patients and contributes to a worse prognosis.[51] In a HFpEF cohort that underwent coronary angiography, patients with CAD (68% of the cohort) showed increased mortality and EF deterioration that was mitigated by revascularization.[52]

Pulmonary hypertension and right ventricular dysfunction

Large community studies demonstrate that pulmonary hypertension is prevalent, often severe and independently predicts mortality in HFpEF.[53] It may discriminate between HFpEF and hypertension suggesting a role in symptom development. Moreover, symptoms develop regardless of capillary wedge pressure, which further insinuates a pre-capillary component.[53] More recently, right ventricular dysfunction was documented in one-third of HFpEF patients undergoing right heart catheterization and shown to be an independent predictor of mortality.[54,55]

Peripheral factors

Effort intolerance, which is the core HF sign and symptom in HFpEF, is not solely due to low cardiovascular reserve but also to poor peripheral oxygen extraction by the skeletal muscle.[56] Indeed, chronotropic incompetence and low systolic reserve lead to further reliance on peripheral oxygen extraction to meet demands in HFpEF but peripheral extraction also fails due to abnormalities of both skeletal muscle and the microvasculature[57] (see supplementary material online, *Figure S1*). Of note, the improvements in exercise capacity due to exercise training appear to derive primarily from improved peripheral (arterial and/or skeletal muscle) function, highlighting the important contribution and plasticity of peripheral factors.[58]

Can we improve characterization of heart failure with preserved ejection fraction phenotypes?

Large cohorts and RCTs have established wide heterogeneity in aetiology, remodelling patterns, stages of presentation, and co-morbid conditions. It has been proposed that patient heterogeneity is in fact a central reason why so many studies had neutral outcomes. A central challenge will thus be to identify relevant subgroups in which specific therapeutic strategies may be tested. A task that will require integration of clinical, structural and functional data.[6,59]

Experimental models

Given its complex pathophysiology, none of the current models fully emulates HFpEF and probably none ever will. Preclinical tests should build upon robust features of each model.[60] A detailed overview of available animal models is provided in the supplementary material online, *Table S1*.

Small animals

Only salt-sensitive rats and obese hypertensive and diabetic ZSF1 rats have a clear demonstration of increased lung weight, which could relate to HF.[61] Salt-sensitive rats however have been criticized because they develop LV dilatation and decreased EF.[62] As for ZSF1 obese rats, they have low peak maximum oxygen consumption and effort intolerance, which puts the model one step ahead towards clinical translation.[63] Additionally, microvascular injury, ischaemia, inflammation and titin hypophosphorylation have been demonstrated.[31,64] ZSF1 obese rats have a convenient hypertensive lean control and mimic many features of HFpEF. Valuable insights into alternatives to effort testing have been proposed.[63] Nevertheless, we must highlight several drawbacks. They are young adults with untreated metabolic syndrome and do not recapitulate the scenario of an elderly patient, they progress to renal failure at an older age, the full-blown phenotype is hard to recapitulate in reproductive age females, and they show only mild extracellular matrix changes. Animal models of pulmonary hypertension associated with HFpEF are also needed, a two-hit model was recently proposed.[65] As for mice, few models have been able to mimic HFpEF. Myosin-binding protein C phosphorylation-deficient mice develop LVH and stiffness, delayed relaxation, lung congestion, and poor spontaneous activity, but unfortunately also slightly depressed EF[66] whereas obese and diabetic $Lepr^{db/db}$ mice develop LV stiffening due mainly to titin hypophosphorylation.[67] The main issues with $Lepr^{db/db}$ are marked changes in metabolism and later decline in EF. Mice models may provide an invaluable contribution to pathophysiological studies by selective genetic manipulation of disease modifiers. Titin immunoglobulin domain-coding exon-deficient mice develop cardiomyocyte stiffening with effort intolerance.[68]

Large animals

Large animal models are highly desirable because they better mimic human physiology. HFpEF has been modelled in old hypertensive dogs by renal wrapping.[69] They show hypertrophy, fibrosis and impaired relaxation. Recently, a new model was developed in young female landrace pigs.[70] Pigs were rendered hypertensive by deoxycorticosterone coupled with a high-salt diet while hyperlipidaemia was induced by a high-cholesterol diet. They did not show increased fibrosis, but did show concentric hypertrophic remodelling and stiffening. Although animals were not symptomatic, disturbances were further aggravated at high pacing rates. Authors attributed their findings to impaired PKG signalling, titin isoform shift and hypophosphorylation.[70] This model has drawbacks such as marked hypercholesterolaemia, a young age and mild hypertension. HFpEF was also mimicked in dogs by repeated coronary microembolization, the only model that partly addresses the link with CAD.[71] Lastly, large animal models are essential for device development and intervention therapies, as recently reported with proof-of-concept percutaneous pericardiotomy in pigs.[72]

Skinned cardiac myocytes

Some of the most influential pathophysiology findings from HFpEF patients have been obtained in skinned cardiomyocyte

preparations.[26] These studies indicate that LV stiffness is mostly ascribed to passive stiffness of the cardiomyocytes themselves. Mechanistically, a shift in titin isoforms and hypophosphorylation was directly implicated in higher passive force. Findings were reproduced in an animal model of HFpEF.[31] Access to HFpEF patients' biopsies is scarce because there is no formal indication for endomyocardial biopsy other than clinical suspicion of restrictive, infiltrative or inflammatory cardiomyopathy. This leads to selection bias, because biopsies are usually collected in younger patients without CAD. Another concern is the source of HFpEF cardiomyocytes usually obtained from the endocardium and their controls usually obtained from transplanted patients' right ventricle or donor hearts. Finally, studies have been performed with expanded lattice spacing at low temperature. Works in intact cardiomyocytes at physiological temperatures show that skinned cardiomyocyte preparations miss the actomyosin contribution.[29] Pros and cons of various experimental set-ups from cell function to *in vivo* cardiovascular function assessment are summarized in *Table 1*.

Given its growing health impact and current lack of evidence-based therapy, it may be justifiable to extend biopsy collection to a broader population of HFpEF patients in experienced centres where reported complication rates are minimal,[73] not only at catheterization laboratories but also in surgical theatres.[74] Whenever possible, findings from cardiomyocyte preparations should be translated to larger set-ups as assessing cardiomyocyte sarcomere shortening, which rarely spans more than 15%, is a poor surrogate of cardiac function, which relies on a complex architecture to achieve EF of over 50%.[75,76]

Intact cardiac myocytes

An important aspect of diastolic function is disturbed intracellular Ca^{2+} and Na^{+} handling. While in HFrEF, decreased amplitudes of cytosolic Ca^{2+} transients play the dominant role for contractile dysfunction, it is less clear to which extent alterations of cytosolic Ca^{2+} (and Na^{+}) handling actually contribute to DD in HFpEF. Cellular ion handling is commonly analysed in isolated, intact cardiac myocytes paced by electrical field stimulation, in particular from animal models. The advantage of these studies over the skinned cardiomyocyte preparations is that, together with cell shortening, cytosolic ion handling and its alterations can be analysed by fluorescent probes, while the skinned myocyte technique only analyses sarcomeric function.

It is currently largely unclear how far mitochondrial dysfunction and/or oxidative stress contribute to diastolic (and/or mild systolic) dysfunction also in HFpEF. Studies on isolated cardiac myocytes allow the investigation of the pyridine nucleotide redox state, membrane potential and reactive oxygen species in mitochondria integrated in their physiological cellular context using fluorescence imaging combined with field stimulation.[77,78]

A drawback of both techniques is that myocytes usually lie slack on a cover slip without any physical workload. This underestimates physiological workload and may have important implications for mitochondrial energetics and excitation–contraction coupling.[79] A major recent advance was the development of techniques that allow stretching and imposition of various degrees of preload and afterload on isolated cardiac myocytes upon attachment to thin glass rods.[29,79]

Langendorff and working intact isolated heart preparations

The Langendorff model can be used to assess end-diastolic pressure–volume relationship and LV stiffness much in the way pressure–volume catheters do *in vivo*, but devoid of systemic effects. Technical challenges have been reviewed.[80] This model complements findings on cell/tissue preparations and constitutes a bridge to whole-heart physiology.[30]

Assessment of heart failure with preserved ejection fraction in experimental models

Haemodynamic evaluation

This approach is invasive, requiring deeper anaesthesia which is usually confined to terminal evaluation. Nonetheless, serial evaluations and telemetry are feasible. Time-honoured approaches have relied on a separate assessment of active relaxation and passive end-diastolic stiffness[9,81] but newer methods, based on global optimization fitting, may be of added value.[82] Methodological aspects of open-chest and closed-chest approaches have been extensively reviewed.[83] Stiffness is usually derived from end-diastolic pressure–volume relationships. In closed-chest preparations the first beats after preload reduction are usually influenced by right ventricular unloading which accounts for almost 30–40% of resting end-diastolic pressure,[9] and this should be accounted for. The LV stiffness constant has units of volume^{-1} and therefore depends on LV geometry and size. Several approaches have been used to circumvent this problem. One approach is to derive stress–strain analogues, including estimates of LV mass or wall thickness,[81] another is to index volumes.[63] Assessment of a single point of end-diastolic pressure and end-diastolic volume is highly load-dependent and inaccurate. Despite its helpful use in large community studies, single beat methods raise concerns in the experimental scenario. Even after pressure correction for the active component of relaxation they are prone to error in individual estimation, particularly in closed-chest preparations.[84] Methods based on volume-normalized data acquired in various species are also better when applied to groups rather than individuals, have bias in the high and low pressure range, and limitations in HFpEF.[85] Lastly, contractile reserve is impaired in HFpEF and should also be appraised in experimental models, sudden afterload unmasks low contractile reserve.[86]

Imaging methods

Non-invasive imaging methods are favoured both in clinical practice and experimental research. Echocardiography is the first choice.[87] Most of the standard clinical approaches can be translated to animal research using high-frequency linear-array probes even with common echocardiography machines.[31,63] The E/E' ratio has been

Table 1 Experimental approaches to assess heart failure with preserved ejection fraction

	Pros	Cons
Haemodynamics	Gold standard to assess LV compliance and relaxation *in vivo*; pharmacological manipulation (heart and vessels)	Requires anaesthesia, surgical preparation and mechanical ventilation; open-chest deviates from physiology; need to optimize fluid replacement
Muscle strip and cardiomyocyte preparations	Assessment of role of excitation–contraction coupling, in particular, cellular ion handling, myofilament function, titin, residual Ca^{2+} and cross-bridges, bioenergetics, and ECM; extensive pharmacological manipulation (myocardium)	Temperature, pH and osmolarity may deviate from physiology; cannot account for cardiac geometry and coronary perfusion; skinned preparations cannot assess membrane-dependent signalling pathways or Ca^{2+} handling
Langendorff and working heart	*Ex vivo* bridge between haemodynamics and muscle strips/cardiomyocytes; working heart allows for better preload control; assess role of coronary perfusion; intrinsic myocardial properties (without anaesthetics, systemic influences, ventricular interdependence and pericardial restraint); pharmacological studies (myocardium and coronaries)	Deviates from *in situ* condition (without pericardium and ventricular interdependence); cannot account for systemic influences
Echocardiography and MRI	Assess flows, dimensions and strain; serial non-invasive evaluation under light sedation; MRI and MRS enable whole-heart evaluation of fibrosis and bioenergetics, respectively; possibility for 3D reconstruction and molecular imaging	Pressures can only be estimated from surrogates; lower time-resolution than pressure measurements
Effort testing	Assess cardiovascular reserve and effort intolerance; treadmill testing with gas analysis ($\dot{V}O_2$max and anaerobic threshold); load, pharmacological or HR manipulations (reasonable substitutes, should be employed in any functional evaluation set-up)	Requires equipment not readily available in many laboratories (treadmill couple to a gas analyser)

ECM, extracellular matrix; HFpEF, heart failure with preserved ejection fraction; HR, heart rate; LV, left ventricular; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; $\dot{V}O_2$max, maximum oxygen consumption.

extensively investigated as a predictor of LV filling pressures, with conflicting results. It should be emphasized that relying on only a single parameter is usually less informative than an integrative analysis.[87] Strain analysis is mainly a research tool, but it has the potential to overcome tissue Doppler imaging. It can gauge all myocardial segments and account for chamber geometry. It remains to be established whether strain-derived echocardiographic parameters may be of added value to HFpEF diagnosis. Speckle tracking has advantages over tissue Doppler imaging, it is unaffected by the angle of alignment but is also constrained by lower time-resolution. In rodents, resolution must be high enough to track speckles and demands high-frequency probes and specialized machines.

Another interesting field of research is the left atrium. Left atrial volume is a sensitive marker of severity and duration of HFpEF and the left atrium actively modulates LV diastolic function[88] but even early disturbances in atrial function may constitute markers of HFpEF.[89] Application of magnetic resonance imaging (MRI) in HFpEF remains limited. Although MRI with through-plane-phase-contrast and myocardial tagging/feature tracking matches most of echocardiography's capabilities,[87] MRI has lower time-resolution and availability, inability to perform real-time measurements, lack of dedicated software for diastolic function, higher costs, and relative contraindications but it excels when the acoustic window is poor, enabling better space-resolution and universal estimation of pulmonary venous flow. MRI has extended possibilities such as the ability to assess interstitial myocardial fibrosis. The most divulged approach is late gadolinium enhancement with T_1-mapping, a technology that has been translated to rodents[90] enabling precise whole-heart quantification with repeat evaluation; the extracellular compartment is likely to play an important pathophysiological role and could be an important surrogate end-point. Another possibility is the assessment of cardiac bioenergetics by way of ^{31}P magnetic resonance spectroscopy.[91]

How to assess heart failure in animal models?

Several animal models have been proposed as HFpEF models without objective evidence of HF. These should be viewed as models of DD but whether it translates into HF is yet to be defined. Lung congestion, as assessed by lung weight, was proposed as a marker of HF.[58] Because lung weights are prone to confounding influences we propose that effort testing should be undertaken and that no model should be presented as HFpEF without clear documentation of effort intolerance.

Exercise testing (diastolic stress tests) and alternatives

Several limitations to cardiovascular reserve have been shown in HFpEF during dynamic exercise but their relative role is disputed.

Table 2 Practical recommendations for translational research on heart failure with preserved ejection fraction

Robust *in vivo* documentation of a heart failure surrogate is warranted to establish any experimental model of HFpEF, we recommend effort testing
Cardiovascular phenotype should be extensively characterized in animal models of HFpEF and preclinical drug testing, we recommend using multiple experimental set-ups from *in vivo* to *in vitro*
Research on the pathophysiological roles and impact in ventricular/vascular structure and function of co-morbidities, ageing and gender should be a priority in available HFpEF models
New developments in imaging methods should continue to be applied to HFpEF experimental models in order to derive non-invasive assessment methodologies
Discovery-driven genomics, transcriptomics and proteomics in HFpEF patient and HFpEF animal model samples and bioinformatics integration with clinical/functional phenotype are warranted
Research on disturbed intercellular communication in HFpEF is a priority, assessment of engineered tissue from patient-derived induced pluripotent stem cells may be a feasible approach
The role played by coronary and systemic microvascular dysfunction and the underlying mechanisms should be explored in HFpEF models
Systemic involvement should be further explored in models of HFpEF, focusing particularly on the adipose tissue, skeletal muscle, nervous system, kidneys and lungs
Collaborations with clinical researchers are the key for clinically oriented research and easy translation of experimental findings

HFpEF, heart failure with preserved ejection fraction.

It must be emphasized that most HFpEF patients are elderly and have various co-morbidities that may preclude dynamic exercise or confound interpretation. Still, a cardiopulmonary exercise test is an invaluable diagnostic tool,[92] measures of submaximal and peak effort were shown to be reliable in HFpEF patients[93] and have been used as strong end-points in RCTs.[94] Stress and effort testing is increasingly advocated also in echocardiography, but it remains unclear which variables should be sought and under which protocol.[95] A reasonable alternative when dynamic exercise is not feasible might be load or pharmacological manipulation. Although it may be technically challenging to have joint hemodynamic evaluation and effort testing with maximum oxygen consumption, such an integrated evaluation of all determinants of effort intolerance could be an important contribution from experimental models of HFpEF. Joint afterload and preload elevation with selective vasopressors is potentially helpful as appraised experimentally.[63] Pacing-induced tachycardia is another alternative, whereas the role of dobutamine is debated, as some studies suggest it may impair E´ response in HFpEF, while others show smaller increases in systolic wall tension than dynamic exercise, even decreasing end-diastolic volume and myocardial oxygen consumption in the hypertrophic heart, without worsening DD.[63]

Gaps in evidence, what do we need for a systems biology approach?

Due to the lack of availability of samples from HFpEF patients and the lag in experimental modelling, our view on cellular and molecular determinants of HFpEF is poorly formed. Ageing and co-morbidities drive microvascular inflammation[32] and myocardial stiffness[31,49] but this may be only one of the contributing processes. Indeed, HFpEF carries an entirely distinct microRNA signature when compared with HFrEF.[96] Studies addressing proteomics of HFpEF and bioinformatics integration with clinical phenotype are lacking. Moreover, it is likely that these pathways will diverge according to underlying co-morbidities. The role of genetic variants is poorly understood and will require large discovery-driven population-based studies such as those undertaken for LVH.[97] Mimicking these gene variants in animal models may provide important clues. The role of oxidative stress, mitochondrial dysfunction and altered bioenergetics in ageing and co-morbidity-induced myocardial remodelling is well established. Benefits of experimental therapies directed at these targets have been reported in hypertension and ageing models[98] but clear documentation in clinical or experimental HFpEF is needed. The role of afterload, loading sequence and reflected waves should be addressed specifically on HFpEF models. Indeed, a thorough characterization of vascular and microvascular function in experimental HFpEF is warranted and will pave the way for preclinical testing. Unveiling the complete pathophysiological scenario, however, will require an in-depth understanding of the cellular disturbances and complex interplay between endothelium, cardiomyocytes, and fibroblasts[99] as well as the high-order interactions with other tissues (see supplementary material online, *Figure S1*). Dysfunctional interactions between cardiac cell-types have been proposed, but while various intercellular communication disturbances have been well documented in related experimental models and cell culture experiments, few were documented in HFpEF.[99] Further developments in co-culture methods and conditioned medium exposure applied to HFpEF samples may provide important clues, as well as research on engineered tissues and human induced pluripotent cell-derived cardiomyocytes.[100] The high-order cross-talk between the heart and lung, skeletal muscle, adipose tissue (AT), and kidney in HF is increasingly acknowledged,[99] but its particular features in HFpEF remain poorly investigated. The lungs are the first organ that are directly affected in HFpEF, pre-capillary and post-capillary components, as well as pulmonary vascular ED have been well established. The potential neuroendocrine contribution of endothelial lung

cells and macrophages to LV disease progression in HFrEF[101] will likely also be involved in HFpEF. Growing evidence that supports a role for lean body mass loss[3] and histopathological and functional disturbances in skeletal muscle biopsies has been documented.[102] Chronic kidney disease is highly prevalent and constitutes a major determinant of outcome in HFpEF, the common link may be ED and inflammation. The interactive role of renal and cardiac disturbances has been recognized in HFpEF models.[103] Finally, the AT may be an important contributor to HFpEF.[104,105] The ageing AT is infiltrated by macrophages and becomes highly active as a source of adipokines/cytokines which may be enhanced by co-morbidities.[106] Adipokines may modulate HFpEF progression. Epicardial AT has been associated with DD in metabolic syndrome. Shared blood supply and neighbourhood epicardial AT may exert important vasocrine and paracrine effects. An important note is its virtual absence in small animals. Finally, a systems biology view of HFpEF will require additional understanding of the roles of ageing and gender in each HFpEF model or experimental set-up.

Conclusions

As the mechanisms and features of HFpEF are progressively unveiled in clinical and epidemiological studies, basic and translational researchers must be aware of its complexity and may profit from guidance both in terms of experimental model and experimental set-up selection as in terms of the most relevant investigation topics. Further access to myocardial samples of representative HFpEF patient cohorts that enable functional and molecular phenotyping is warranted. Animal models should be selected based upon their specific features, favouring those that have effort intolerance or impaired cardiovascular reserve as a clinical counterpart, at least for preclinical therapy testing. The roles of ageing, gender, and specific co-morbidities in HFpEF progression have been poorly explored and should be addressed in these experimental models. Functional evaluation should rely on multiple experimental set-ups, ideally ranging from *in vivo* organ function to *ex vivo* or *in vitro* cell function tests. To attain a systems biology outlook of HFpEF, systemic influences and higher-order cross-talk with other organs should be further investigated, as well as disturbed intercellular communication mechanisms within the heart. Practical recommendations for translational researchers on HFpEF are provided in *Table 2*.

Supplementary Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Systemic changes and intercellular cardiac communication disturbances underlying heart failure with preserved ejection fraction (HFpEF).

Table S1. HFpEF and HFpEF-related experimental models.

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References

1. Cleland JG, Pellicori P, Dierckx R. clinical trials in patients with heart failure and preserved left ventricular ejection fraction. *Heart Fail Clin* 2014;**10**:511–523.
2. Vaduganathan M, Michel A, Hall K, Mulligan C, Nodari S, Shah SJ, Senni M, Triggiani M, Butler J, Gheorghiade M. Spectrum of epidemiological and clinical findings in patients with heart failure with preserved ejection fraction stratified by study design: a systematic review. *Eur J Heart Fail* 2016;**18**:54–65.
3. Kitzman DW, Brubaker P, Morgan T, Haykowsky M, Hundley G, Kraus WE, Eggebeen J, Nicklas BJ. Effect of caloric restriction or aerobic exercise training on peak oxygen consumption and quality of life in obese older patients with heart failure with preserved ejection fraction: a randomized clinical trial. *JAMA* 2016;**315**:36–46.
4. Edelmann F, Gelbrich G, Dungen HD, Frohling S, Wachter R, Stahrenberg R, Binder L, Topper A, Lashki DJ, Schwarz S, Herrmann-Lingen C, Loffler M, Hasenfuss G, Halle M, Pieske B. Exercise training improves exercise capacity and diastolic function in patients with heart failure with preserved ejection fraction: results of the Ex-DHF (Exercise training in Diastolic Heart Failure) pilot study. *J Am Coll Cardiol* 2011;**58**:1780–1791.
5. De Keulenaer GW, Segers VF, Zannad F, Brutsaert DL. The future of pleiotropic therapy in heart failure. Lessons from the benefits of exercise training on endothelial function. *Eur J Heart Fail* 2017; **9**:603–614.
6. Shah SJ. Matchmaking for the optimization of clinical trials of heart failure with preserved ejection fraction: no laughing matter. *J Am Coll Cardiol* 2013;**62**:1339–1342.
7. Roh J, Houstis N, Rosenzweig A. Why don't we have proven treatments for HFpEF? *Circ Res* 2017;**120**:1243–1245.
8. van Riet EE, Hoes AW, Wagenaar KP, Limburg A, Landman MA, Rutten FH. Epidemiology of heart failure: the prevalence of heart failure and ventricular dysfunction in older adults over time. A systematic review. *Eur J Heart Fail* 2016;**18**:242–252.
9. Kass DA. Assessment of diastolic dysfunction. Invasive modalities. *Cardiol Clin* 2000;**18**:571–586.

10. Redfield MM, Jacobsen SJ, Burnett, Jr JC, Mahoney DW, Bailey KR, Rodeheffer RJ. Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. *JAMA* 2003;**289**:194–202.
11. Kane GC, Karon BL, Mahoney DW, Redfield MM, Roger VL, Burnett JC Jr, Jacobsen SJ, Rodeheffer RJ. Progression of left ventricular diastolic dysfunction and risk of heart failure. *JAMA* 2011;**306**:856–863.
12. Paulus WJ, Tschope C, Sanderson JE, Rusconi C, Flachskampf FA, Rademakers FE, Marino P, Smiseth OA, De Keulenaer G, Leite-Moreira AF, Borbely A, Edes I, Handoko ML, Heymans S, Pezzali N, Pieske B, Dickstein K, Fraser AG, Brutsaert DL. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. *Eur Heart J* 2007;**28**:2539–2550.
13. Borlaug BA, Nishimura RA, Sorajja P, Lam CS, Redfield MM. Exercise hemodynamics enhance diagnosis of early heart failure with preserved ejection fraction. *Circ Heart Fail* 2010;**3**:588–595.
14. Obokata M, Kane GC, Reddy YN, Olson TP, Melenovsky V, Borlaug BA. Role of diastolic stress testing in the evaluation for heart failure with preserved ejection fraction: a simultaneous invasive-echocardiographic study. *Circulation* 2017 28;**135**:825–838.
15. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, Falk V, Gonzalez-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GM, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail* 2016;**18**:891–975.
16. Shah AM, Claggett B, Sweitzer NK, Shah SJ, Anand IS, O'Meara E, Desai AS, Heitner JF, Li G, Fang J, Rouleau J, Zile MR, Markov V, Ryabov V, Reis G, Assmann SF, McKinlay SM, Pitt B, Pfeffer MA, Solomon SD. Cardiac structure and function and prognosis in heart failure with preserved ejection fraction: findings from the echocardiographic study of the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist (TOPCAT) Trial. *Circ Heart Fail* 2014;**7**:740–751.
17. Persson H, Lonn E, Edner M, Baruch L, Lang CC, Morton JJ, Ostergren J, McKelvie RS. Diastolic dysfunction in heart failure with preserved systolic function: need for objective evidence: results from the CHARM Echocardiographic Substudy-CHARMES. *J Am Coll Cardiol* 2007;**49**:687–694.
18. Borlaug BA, Olson TP, Lam CS, Flood KS, Lerman A, Johnson BD, Redfield MM. Global cardiovascular reserve dysfunction in heart failure with preserved ejection fraction. *J Am Coll Cardiol* 2010;**56**:845–854.
19. Borlaug BA. The pathophysiology of heart failure with preserved ejection fraction. *Nat Rev Cardiol* 2014;**11**:507–515.
20. Maciver DH, Townsend M. A novel mechanism of heart failure with normal ejection fraction. *Heart* 2008;**94**:446–449.
21. Borlaug BA, Lam CS, Roger VL, Rodeheffer RJ, Redfield MM. Contractility and ventricular systolic stiffening in hypertensive heart disease insights into the pathogenesis of heart failure with preserved ejection fraction. *J Am Coll Cardiol* 2009;**54**:410–418.
22. Abudiab MM, Redfield MM, Melenovsky V, Olson TP, Kass DA, Johnson BD, Borlaug BA. Cardiac output response to exercise in relation to metabolic demand in heart failure with preserved ejection fraction. *Eur J Heart Fail* 2013;**15**:776–785.
23. Dunlay SM, Roger VL, Weston SA, Jiang R, Redfield MM. Longitudinal changes in ejection fraction in heart failure patients with preserved and reduced ejection fraction. *Circ Heart Fail* 2012;**5**:720–726.
24. Bhella PS, Pacini EL, Prasad A, Hastings JL, Adams-Huet B, Thomas JD, Grayburn PA, Levine BD. Echocardiographic indices do not reliably track changes in left-sided filling pressure in healthy subjects or patients with heart failure with preserved ejection fraction. *Circ Cardiovasc Imaging* 2011;**4**:482–489.
25. Penicka M, Bartunek J, Trakalova H, Hrabakova H, Maruskova M, Karasek J, Kocka V. Heart failure with preserved ejection fraction in outpatients with unexplained dyspnea: a pressure-volume loop analysis. *J Am Coll Cardiol* 2010;**55**:1701–1710.
26. van Heerebeek L, Borbely A, Niessen HW, Bronzwaer JG, van der Velden J, Stienen GJ, Linke WA, Laarman GJ, Paulus WJ. Myocardial structure and function differ in systolic and diastolic heart failure. *Circulation* 2006;**113**:1966–1973.
27. Chung CS, Methawasin M, Nelson OL, Radke MH, Hidalgo CG, Gotthardt M, Granzier HL. Titin based viscosity in ventricular physiology: an integrative investigation of PEVK-actin interactions. *J Mol Cell Cardiol* 2011;**51**:428–434.
28. Hamdani N, Paulus WJ, van Heerebeek L, Borbely A, Boontje NM, Zuidwijk MJ, Bronzwaer JG, Simonides WS, Niessen HW, Stienen GJ, van der Velden J. Distinct myocardial effects of beta-blocker therapy in heart failure with normal and reduced left ventricular ejection fraction. *Eur Heart J* 2009;**30**:1863–1872.
29. King NM, Methawasin M, Nedrud J, Harrell N, Chung CS, Helmes M, Granzier H. Mouse intact cardiac myocyte mechanics: cross-bridge and titin-based stress in unactivated cells. *J Gen Physiol* 2011;**137**:81–91.
30. Sequeira V, Najafi A, McConnell M, Fowler ED, Bollen IA, Wust RC, dos Remedios C, Helmes M, White E, Stienen GJ, Tardiff J, Kuster DW, van der Velden J. Synergistic role of ADP and Ca^{2+} in diastolic myocardial stiffness. *J Physiol* 2015;**593**:3899–3916.
31. Hamdani N, Franssen C, Lourenco A, Falcao-Pires I, Fontoura D, Leite S, Plettig L, Lopez B, Ottenheijm CA, Becher PM, Gonzalez A, Tschope C, Diez J, Linke WA, Leite-Moreira AF, Paulus WJ. Myocardial titin hypophosphorylation importantly contributes to heart failure with preserved ejection fraction in a rat metabolic risk model. *Circ Heart Fail* 2013;**6**:1239–1249.
32. Mohammed SF, Hussain S, Mirzoyev SA, Edwards WD, Maleszewski JJ, Redfield MM. Coronary microvascular rarefaction and myocardial fibrosis in heart failure with preserved ejection fraction. *Circulation* 2015;**131**:550–559.
33. Loffredo FS, Nikolova AP, Pancoast JR, Lee RT. Heart failure with preserved ejection fraction: molecular pathways of the aging myocardium. *Circ Res* 2014;**115**:97–107.
34. O'Rourke MF, Safar ME, Dzau V. The Cardiovascular Continuum extended: aging effects on the aorta and microvasculature. *Vasc Med* 2010;**15**:461–468.
35. Chirinos JA, Segers P, Gupta AK, Swillens A, Rietzschel ER, De Buyzere ML, Kirkpatrick JN, Gillebert TC, Wang Y, Keane MG, Townsend R, Ferrari VA, Wiegers SE, St John Sutton M. Time-varying myocardial stress and systolic pressure-stress relationship: role in myocardial-arterial coupling in hypertension. *Circulation* 2009;**119**:2798–2807.
36. Chirinos JA, Segers P, Rietzschel ER, De Buyzere ML, Raja MW, Claessens T, De Bacquer D, St John Sutton M, Gillebert TC. Early and late systolic wall stress differentially relate to myocardial contraction and relaxation in middle-aged adults: the Asklepios study. *Hypertension* 2013;**61**:296–303.
37. Weber T, Wassertheurer S, Rammer M, Haiden A, Hametner B, Eber B. Wave reflections, assessed with a novel method for pulse wave separation, are associated with end-organ damage and clinical outcomes. *Hypertension* 2012;**60**:534–541.
38. Reddy YNV, Andersen MJ, Obokata M, Koepp KE, Kane GC, Melenovsky V, Olson TP, Borlaug BA. Arterial stiffening with exercise in patients with heart failure and preserved ejection fraction. *J Am Coll Cardiol* 2017;**70**:136–148.
39. Mohammed SF, Borlaug BA, Roger VL, Mirzoyev SA, Rodeheffer RJ, Chirinos JA, Redfield MM. Comorbidity and ventricular and vascular structure and function in heart failure with preserved ejection fraction: a community-based study. *Circ Heart Fail* 2012;**5**:710–719.
40. Redfield MM, Jacobsen SJ, Borlaug BA, Rodeheffer RJ, Kass DA. Age- and gender-related ventricular–vascular stiffening: a community-based study. *Circulation* 2005;**112**:2254–2262.
41. Zhao Z, Wang H, Jessup JA, Lindsey SH, Chappell MC, Groban L. Role of estrogen in diastolic dysfunction. *Am J Phys Heart Circ Physiol* 2014;**306**:H628–H640.
42. Mentz RJ, Kelly JP, von Lueder TG, Voors AA, Lam CS, Cowie MR, Kjeldsen K, Jankowska EA, Atar D, Butler J, Fiuzat M, Zannad F, Pitt B, O'Connor CM. Noncardiac comorbidities in heart failure with reduced versus preserved ejection fraction. *J Am Coll Cardiol* 2014;**64**:2281–2293.
43. Liu M, Fang F, Yu CM. Noncardiac comorbidities in heart failure with preserved ejection fraction – commonly ignored fact. *Circ J* 2015;**79**:954–959.
44. Ter Maaten JM, Damman K, Verhaar MC, Paulus WJ, Duncker DJ, Cheng C, van Heerebeek L, Hillege HL, Lam CS, Navis G, Voors AA. Connecting heart failure with preserved ejection fraction and renal dysfunction: the role of endothelial dysfunction and inflammation. *Eur J Heart Fail* 2016;**18**:588–598.
45. Ather S, Chan W, Bozkurt B, Aguilar D, Ramasubbu K, Zachariah AA, Wehrens XH, Deswal A. Impact of noncardiac comorbidities on morbidity and mortality in a predominantly male population with heart failure and preserved versus reduced ejection fraction. *J Am Coll Cardiol* 2012;**59**:998–1005.
46. Zile MR, Gaasch WH, Anand IS, Haass M, Little WC, Miller AB, Lopez-Sendon J, Teerlink JR, White M, McMurray JJ, Komajda M, McKelvie R, Ptaszynska A, Hetzel SJ, Massie BM, Carson PE; I-Preserve Investigators. Mode of death in patients with heart failure and a preserved ejection fraction: results from the Irbesartan in Heart Failure With Preserved Ejection Fraction Study (I-Preserve) trial. *Circulation* 2010;**121**:1393–1405.
47. Berry SD, Ngo L, Samelson EJ, Kiel DP. Competing risk of death: an important consideration in studies of older adults. *J Am Geriatr Soc* 2010;**58**:783–787.
48. Falcao-Pires I, Leite-Moreira AF. Diabetic cardiomyopathy: understanding the molecular and cellular basis to progress in diagnosis and treatment. *Heart Fail Rev* 2012;**17**:325–344.
49. Paulus WJ, Tschope C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. *J Am Coll Cardiol* 2013;**62**:263–271.

50. Hoenig MR, Bianchi C, Rosenzweig A, Sellke FW. The cardiac microvasculature in hypertension, cardiac hypertrophy and diastolic heart failure. *Curr Vasc Pharmacol* 2008;**6**:292–300.
51. Shah SJ. Evolving approaches to the management of heart failure with preserved ejection fraction in patients with coronary artery disease. *Curr Treat Options Cardiovasc Med* 2010;**12**:58–75.
52. Hwang SJ, Melenovsky V, Borlaug BA. Implications of coronary artery disease in heart failure with preserved ejection fraction. *J Am Coll Cardiol* 2014;**63**(25 Pt A):2817–2827.
53. Lam CS, Roger VL, Rodeheffer RJ, Borlaug BA, Enders FT, Redfield MM. Pulmonary hypertension in heart failure with preserved ejection fraction: a community-based study. *J Am Coll Cardiol* 2009;**53**:1119–1126.
54. Melenovsky V, Hwang SJ, Lin G, Redfield MM, Borlaug BA. Right heart dysfunction in heart failure with preserved ejection fraction. *Eur Heart J* 2014;**35**:3452–3462.
55. Gorter TM, Hoendermis ES, van Veldhuisen DJ, Voors AA, Lam CS, Geelhoed B, Willems TP, van Melle JP. Right ventricular dysfunction in heart failure with preserved ejection fraction: a systematic review and meta-analysis. *Eur J Heart Fail* 2016;**18**:1472–1487.
56. Haykowsky MJ, Brubaker PH, John JM, Stewart KP, Morgan TM, Kitzman DW. Determinants of exercise intolerance in elderly heart failure patients with preserved ejection fraction. *J Am Coll Cardiol* 2011;**58**:265–274.
57. Dhakal BP, Malhotra R, Murphy RM, Pappagianopoulos PP, Baggish AL, Weiner RB, Houstis NE, Eisman AS, Hough SS, Lewis GD. Mechanisms of exercise intolerance in heart failure with preserved ejection fraction: the role of abnormal peripheral oxygen extraction. *Circ Heart Fail* 2015;**8**:286–294.
58. Haykowsky MJ, Brubaker PH, Stewart KP, Morgan TM, Eggebeen J, Kitzman DW. Effect of endurance training on the determinants of peak exercise oxygen consumption in elderly patients with stable compensated heart failure and preserved ejection fraction. *J Am Coll Cardiol* 2012;**60**:120–128.
59. D'Elia E, Vaduganathan M, Gori M, Gavazzi A, Butler J, Senni M. Role of biomarkers in cardiac structure phenotyping in heart failure with preserved ejection fraction: critical appraisal and practical use. *Eur J Heart Fail* 2015;**17**:1231–1239.
60. Conceicao G, Heinonen I, Lourenco AP, Duncker DJ, Falcao-Pires I. Animal models of heart failure with preserved ejection fraction. *Neth Heart J* 2016;**24**:275–286.
61. Horgan S, Watson C, Glezeva N, Baugh J. Murine models of diastolic dysfunction and heart failure with preserved ejection fraction. *J Card Fail* 2014;**20**:984–995.
62. Klotz S, Hay I, Zhang G, Maurer M, Wang J, Burkhoff D. Development of heart failure in chronic hypertensive Dahl rats: focus on heart failure with preserved ejection fraction. *Hypertension* 2006;**47**:901–911.
63. Leite S, Oliveira-Pinto J, Tavares-Silva M, Abdellatif M, Fontoura D, Falcao-Pires I, Leite-Moreira AF, Lourenco AP. Echocardiography and invasive hemodynamics during stress testing for diagnosis of heart failure with preserved ejection fraction: an experimental study. *Am J Phys Heart Circ Physiol* 2015;**308**:H1556–H1563.
64. Tofovic SP, Salah EM, Smits GJ, Whalley ET, Ticho B, Deykin A, Jackson EK. Dual A1/A2B receptor blockade improves cardiac and renal outcomes in a rat model of heart failure with preserved ejection fraction. *J Pharm Exp Ther* 2016;**356**:333–340.
65. Lai YC, Tabima DM, Dube JJ, Hughan KS, Vanderpool RR, Goncharov DA, St. Croix CM, Garcia-Ocanã A, Goncharova EA, Tofovic SP, Mora AL, Gladwin MT. SIRT3-AMP-activated protein kinase activation by nitrite and metformin improves hyperglycemia and normalizes pulmonary hypertension associated with heart failure with preserved ejection fraction. *Circulation* 2016;**133**:717–731.
66. Rosas PC, Liu Y, Abdalla MI, Thomas CM, Kidwell DT, Dusio GF, Mukhopadhyay D, Kumar R, Baker KM, Mitchell BM, Powers PA, Fitzsimons DP, Patel BG, Warren CM, Solaro RJ, Moss RL, Tong CW. Phosphorylation of cardiac myosin-binding protein-C is a critical mediator of diastolic function. *Circ Heart Fail* 2015;**8**:582–594.
67. Hamdani N, Hervent AS, Vandekerckhove L, Matheeussen V, Demolder M, Baerts L, De Meester I, Linke WA, Paulus WJ, De Keulenaer GW. Left ventricular diastolic dysfunction and myocardial stiffness in diabetic mice is attenuated by inhibition of dipeptidyl peptidase 4. *Cardiovasc Res* 2014;**104**:423–431.
68. Chung CS, Hutchinson KR, Methawasin M, Saripalli C, Smith JE, 3rd, Hidalgo CG, Luo X, Labeit S, Guo C, Granzier HL. Shortening of the elastic tandem immunoglobulin segment of titin leads to diastolic dysfunction. *Circulation* 2013;**128**:19–28.
69. Munagala VK, Hart CY, Burnett JC, Jr., Meyer DM, Redfield MM. Ventricular structure and function in aged dogs with renal hypertension: a model of experimental diastolic heart failure. *Circulation* 2005;**111**:1128–1135.
70. Schwarzl M, Hamdani N, Seiler S, Alogna A, Manninger M, Reilly S, Zirngast B, Kirsch A, Steendijk P, Verderber J, Zweiker D, Eller P, Hofler G, Schauer S, Eller K, Maechler H, Pieske BM, Linke WA, Casadei B, Post H. A porcine model of hypertensive cardiomyopathy: implications for heart failure with preserved ejection fraction. *Am J Phys Heart Circ Physiol* 2015;**309**:H1407–H1418.
71. He KL, Dickstein M, Sabbah HN, Yi GH, Gu A, Maurer M, Wei CM, Wang J, Burkhoff D. Mechanisms of heart failure with well preserved ejection fraction in dogs following limited coronary microembolization. *Cardiovasc Res* 2004;**64**:72–83.
72. Borlaug BA, Carter RE, Melenovsky V, DeSimone CV, Gaba P, Killu A, Naksuk N, Lerman L, Asirvatham SJ. Percutaneous pericardial resection: a novel potential treatment for heart failure with preserved ejection fraction. *Circ Heart Fail* 2017;**10**:e003612.
73. Chimenti C, Frustaci A. Contribution and risks of left ventricular endomyocardial biopsy in patients with cardiomyopathies: a retrospective study over a 28-year period. *Circulation* 2013;**128**:1531–1541.
74. Zile MR, Baicu CF, Ikonomidis JS, Stroud RE, Nietert PJ, Bradshaw AD, Slater R, Palmer BM, Van Buren P, Meyer M, Redfield MM, Bull DA, Granzier HL, LeWinter MM. Myocardial stiffness in patients with heart failure and a preserved ejection fraction: contributions of collagen and titin. *Circulation* 2015;**131**:1247–1259.
75. Yip GW, Frenneaux M, Sanderson JE. Heart failure with a normal ejection fraction: new developments. *Heart* 2009;**95**:1549–1552.
76. Torrent-Guasp F, Kocica MJ, Corno AF, Komeda M, Carreras-Costa F, Flotats A, Cosin-Aguillar J, Wen H. Towards new understanding of the heart structure and function. *Eur J Cardiothorac Surg* 2005;**27**:191–201.
77. Kohlhaas M, Liu T, Knopp A, Zeller T, Ong MF, Bohm M, O'Rourke B, Maack C. Elevated cytosolic Na+ increases mitochondrial formation of reactive oxygen species in failing cardiac myocytes. *Circulation* 2010;**121**:1606–1613.
78. Maack C, O'Rourke B. Excitation-contraction coupling and mitochondrial energetics. *Basic Res Cardiol* 2007;**102**:369–392.
79. Jian Z, Han H, Zhang T, Puglisi J, Izu LT, Shaw JA, Onofiok E, Erickson JR, Chen YJ, Horvath B, Shimkunas R, Xiao W, Li Y, Pan T, Chan J, Banyasz T, Tardiff JC, Chiamvimonvat N, Bers DM, Lam KS, Chen-Izu Y. Mechanochemotransduction during cardiomyocyte contraction is mediated by localized nitric oxide signaling. *Sci Signal* 2014;**7**:ra27.
80. Liao R, Podesser BK, Lim CC. The continuing evolution of the Langendorff and ejecting murine heart: new advances in cardiac phenotyping. *Am J Phys Heart Circ Physiol* 2012;**303**:H156–H167.
81. Burkhoff D, Mirsky I, Suga H. Assessment of systolic and diastolic ventricular properties via pressure-volume analysis: a guide for clinical, translational, and basic researchers. *Am J Phys Heart Circ Physiol* 2005;**289**:H501–512.
82. Bermejo J, Yotti R, Perez del Villar C, del Alamo JC, Rodriguez-Perez D, Martinez-Legazpi P, Benito Y, Antoranz JC, Desco MM, Gonzalez-Mansilla A, Barrio A, Elizaga J, Fernandez-Aviles F. Diastolic chamber properties of the left ventricle assessed by global fitting of pressure-volume data: improving the gold standard of diastolic function. *J Appl Physiol* 2013;**115**:556–568.
83. Lourenço AP, Falcão-Pires I, Leite-Moreira AF. *In vivo* experimental assessment of cardiac function. In: Cokkinos DV (ed.) *Introduction to Translational Cardiovascular Research*. Cham: Springer International Publishing; 2015. pp. 389–411.
84. Jaber WA, Lam CS, Meyer DM, Redfield MM. Revisiting methods for assessing and comparing left ventricular diastolic stiffness: impact of relaxation, external forces, hypertrophy, and comparators. *Am J Phys Heart Circ Physiol* 2007;**293**:H2738–2746.
85. Klotz S, Dickstein ML, Burkhoff D. A computational method of prediction of the end-diastolic pressure–volume relationship by single beat. *Nat Protoc* 2007;**2**:2152–2158.
86. Leite S, Rodrigues S, Tavares-Silva M, Oliveira-Pinto J, Alaa M, Abdellatif M, Fontoura D, Falcao-Pires I, Gillebert TC, Leite-Moreira AF, Lourenco AP. Afterload-induced diastolic dysfunction contributes to high filling pressures in experimental heart failure with preserved ejection fraction. *Am J Phys Heart Circ Physiol* 2015;**309**:H1648–H1654.
87. Flachskampf FA, Biering-Sorensen T, Solomon SD, Duvernoy O, Bjerner T, Smiseth OA. Cardiac imaging to evaluate left ventricular diastolic function. *JACC Cardiovasc Imaging* 2015;**8**:1071–1093.
88. Melenovsky V, Hwang SJ, Redfield MM, Zakeri R, Lin G, Borlaug BA. Left atrial remodeling and function in advanced heart failure with preserved or reduced ejection fraction. *Circ Heart Fail* 2015;**8**:295–303.
89. Zakeri R, Moulay G, Chai Q, Ogut O, Hussain S, Takahama H, Lu T, Wang XL, Linke WA, Lee HC, Redfield MM. Left atrial remodeling and atrioventricular coupling in a canine model of early heart failure with preserved ejection fraction. *Circ Heart Fail* 2016;**9**:e003238.
90. Stuckey DJ, McSweeney SJ, Thin MZ, Habib J, Price AN, Fiedler LR, Gsell W, Prasad SK, Schneider MD. T_1 mapping detects pharmacological retardation of diffuse cardiac fibrosis in mouse pressure-overload hypertrophy. *Circ Cardiovasc Imaging* 2014;**7**:240–249.

91. Phan TT, Abozguia K, Nallur Shivu G, Mahadevan G, Ahmed I, Williams L, Dwivedi G, Patel K, Steendijk P, Ashrafian H, Henning A, Frenneaux M. Heart failure with preserved ejection fraction is characterized by dynamic impairment of active relaxation and contraction of the left ventricle on exercise and associated with myocardial energy deficiency. *J Am Coll Cardiol* 2009;**54**:402–409.
92. Guazzi M, Arena R, Halle M, Piepoli MF, Myers J, Lavie CJ. 2016 focused update: clinical recommendations for cardiopulmonary exercise testing data assessment in specific patient populations. *Eur Heart J* 2016;**133**:e694–711.
93. Scott JM, Haykowsky MJ, Eggebeen J, Morgan TM, Brubaker PH, Kitzman DW. Reliability of peak exercise testing in patients with heart failure with preserved ejection fraction. *Am J Cardiol* 2012;**110**:1809–1813.
94. Redfield MM, Chen HH, Borlaug BA, Semigran MJ, Lee KL, Lewis G, LeWinter MM, Rouleau JL, Bull DA, Mann DL, Deswal A, Stevenson LW, Givertz MM, Ofili EO, O'Connor CM, Felker GM, Goldsmith SR, Bart BA, McNulty SE, Ibarra JC, Lin G, Oh JK, Patel MR, Kim RJ, Tracy RP, Velazquez EJ, Anstrom KJ, Hernandez AF, Mascette AM, Braunwald E. Effect of phosphodiesterase-5 inhibition on exercise capacity and clinical status in heart failure with preserved ejection fraction: a randomized clinical trial. *JAMA* 2013;**309**:1268–1277.
95. Erdei T, Aakhus S, Marino P, Paulus WJ, Smiseth OA, Fraser AG. Pathophysiological rationale and diagnostic targets for diastolic stress testing. *Heart* 2015;**101**:1355–1360.
96. Watson CJ, Gupta SK, O'Connell E, Thum S, Glezeva N, Fendrich J, Gallagher J, Ledwidge M, Grote-Levi L, McDonald K, Thum T. MicroRNA signatures differentiate preserved from reduced ejection fraction heart failure. *Eur J Heart Fail* 2015;**17**:405–415.
97. van der Harst P, van Setten J, Verweij N, Vogler G, Franke L, Maurano MT, Wang X, Mateo Leach I, Eijgelsheim M, Sotoodehnia N, Hayward C, Sorice R, Meirelles O, Lyytikainen LP, Polasek O, Tanaka T, Arking DE, Ulivi S, Trompet S, Muller-Nurasyid M, Smith AV, Dorr M, Kerr KF, Magnani JW, Del Greco MF, Zhang W, Nolte IM, Silva CT, Padmanabhan S, Tragante V, Esko T, Abecasis GR, Adriaens ME, Andersen K, Barnett P, Bis JC, Bodmer R, Buckley BM, Campbell H, Cannon MV, Chakravarti A, Chen LY, Delitala A, Devereux RB, Doevendans PA, Dominiczak AF, Ferrucci L, Ford I, Gieger C, Harris TB, Haugen E, Heinig M, Hernandez DG, Hillege HL, Hirschhorn JN, Hofman A, Hubner N, Hwang SJ, Iorio A, Kahonen M, Kellis M, Kolcic I, Kooner IK, Kooner JS, Kors JA, Lakatta EG, Lage K, Launer LJ, Levy D, Lundby A, Macfarlane PW, May D, Meitinger T, Metspalu A, Nappo S, Naitza S, Neph S, Nord AS, Nutile T, Okin PM, Olsen JV, Oostra BA, Penninger JM, Pennacchio LA, Pers TH, Perz S, Peters A, Pinto YM, Pfeufer A, Pilia MG, Pramstaller PP, Prins BP, Raitakari OT, Raychaudhuri S, Rice KM, Rossin EJ, Rotter JI, Schafer S, Schlessinger D, Schmidt CO, Sehmi J, Sillje HH, Sinagra G, Sinner MF, Slowikowski K, Soliman EZ, Spector TD, Spiering W, Stamatoyannopoulos JA, Stolk RP, Strauch K, Tan ST, Tarasov KV, Trinh B, Uitterlinden AG, van den Boogaard M, van Duijn CM, van Gilst WH, Viikari JS, Visscher PM, Vitart V, Volker U, Waldenberger M, Weichenberger CX, Westra HJ, Wijmenga C, Wolffenbuttel BH, Yang J, Bezzina CR, Munroe PB, Snieder H, Wright AF, Rudan I, Boyer LA, Asselbergs FW, van Veldhuisen DJ, Stricker BH, Psaty BM, Ciullo M, Sanna S, Lehtimaki T, Wilson JF, Bandinelli S, Alonso A, Gasparini P, Jukema JW, Kaab S, Gudnason V, Felix SB, Heckbert SR, de Boer RA, Newton-Cheh C, Hicks AA, Chambers JC, Jamshidi Y, Visel A, Christoffels VM, Isaacs A, Samani NJ, de Bakker PI. 52 Genetic loci influencing myocardial mass. *J Am Coll Cardiol* 2016;**68**:1435–1448.
98. Eisenberg T, Abdellatif M, Schroeder S, Primessnig U, Stekovic S, Pendl T, Harger A, Schipke J, Zimmermann A, Schmidt A, Tong M, Ruckenstuhl C, Dammbrueck C, Gross AS, Herbst V, Magnes C, Trausinger G, Narath S, Meinitzer A, Hu Z, Kirsch A, Eller K, Carmona-Gutierrez D, Buttner S, Pietrocola F, Knittelfelder O, Schrepfer E, Rockenfeller P, Simonini C, Rahn A, Horsch M, Moreth K, Beckers J, Fuchs H, Gailus-Durner V, Neff F, Janik D, Rathkolb B, Rozman J, de Angelis MH, Moustafa T, Haemmerle G, Mayr M, Willeit P, von Frieling-Salewsky M, Pieske B, Scorrano L, Pieber T, Pechlaner R, Willeit J, Sigrist SJ, Linke WA, Muhlfeld C, Sadoshima J, Dengjel J, Kiechl S, Kroemer G, Sedej S, Madeo F. Cardioprotection and lifespan extension by the natural polyamine spermidine. *Nat Med* 2016;**22**:1428–1438.
99. Bang C, Antoniades C, Antonopoulos AS, Eriksson U, Franssen C, Hamdani N, Lehmann L, Moessinger C, Mongillo M, Muhl L, Speer T, Thum T. Intercellular communication lessons in heart failure. *Eur J Heart Fail* 2015;**17**:1091–1103.
100. Kriegel AJ, Gartz M, Afzal MZ, de Lange WJ, Ralphe JC, Strande JL. Molecular approaches in HFpEF: microRNAs and iPSC-derived cardiomyocytes. *J Cardiovasc Transl Res* 2016;**10**:295–304.
101. Oie E, Ahmed MS, Ueland T, Sikkeland LI, Dahl CP, Hagelin EM, von Lueder T, Edvardsen T, Andreassen AK, Gullestad L, Aukrust P, Yndestad A, Vinge LE, Attramadal H. Adrenomedullin is increased in alveolar macrophages and released from the lungs into the circulation in severe heart failure. *Basic Res Cardiol* 2010;**105**:89–98.
102. Kitzman DW, Nicklas B, Kraus WE, Lyles MF, Eggebeen J, Morgan TM, Haykowsky M. Skeletal muscle abnormalities and exercise intolerance in older patients with heart failure and preserved ejection fraction. *Am J Phys Heart Circ Physiol* 2014;**306**:H1364–H1370.
103. van Dijk CG, Oosterhuis NR, Xu YJ, Brandt M, Paulus WJ, van Heerebeek L, Duncker DJ, Verhaar MC, Fontoura D, Lourenco AP, Leite-Moreira AF, Falcao-Pires I, Joles JA, Cheng C. Distinct endothelial cell responses in the heart and kidney microvasculature characterize the progression of heart failure with preserved ejection fraction in the obese ZSF1 rat with cardiorenal metabolic syndrome. *Circ Heart Fail* 2016;**9**:e002760.
104. Obokata M, Reddy YNV, Pislaru SV, Melenovsky V, Borlaug BA. Evidence Supporting the existence of a distinct obese phenotype of heart failure with preserved ejection fraction. *Circulation* 2017;**136**:6–19.
105. Kitzman DW, Shah SJ. The HFpEF obesity phenotype: the elephant in the room. *J Am Coll Cardiol* 2016;**68**:200–203.
106. Falcao-Pires I, Castro-Chaves P, Miranda-Silva D, Lourenco AP, Leite-Moreira AF. Physiological, pathological and potential therapeutic roles of adipokines. *Drug Discovery Today* 2012;**17**:880–889.