



# Mechanisms underlying the pathophysiology of heart failure with preserved ejection fraction: the tip of the iceberg

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## Abstract

Heart failure with preserved ejection fraction (HFpEF) is a multifaceted syndrome with a complex aetiology often associated with several comorbidities, such as left ventricle pressure overload, diabetes mellitus, obesity, and kidney disease. Its pathophysiology remains obscure mainly due to the complex phenotype induced by all these associated comorbidities and to the scarcity of animal models that adequately mimic HFpEF. Increased oxidative stress, inflammation, and endothelial dysfunction are currently accepted as key players in HFpEF pathophysiology. However, we have just started to unveil HFpEF complexity and the role of calcium handling, energetic metabolism, and mitochondrial function remain to clarify. Indeed, the enlightenment of such cellular and molecular mechanisms represents an opportunity to develop novel therapeutic approaches and thus to improve HFpEF treatment options. In the last decades, the number of research groups dedicated to studying HFpEF has increased, denoting the importance and the magnitude achieved by this syndrome. In the current technological and web world, the amount of information is overwhelming, driving us not only to compile the most relevant information about the theme but also to explore beyond the tip of the iceberg. Thus, this review aims to encompass the most recent knowledge related to HFpEF or HFpEF-associated comorbidities, focusing mainly on myocardial metabolism, oxidative stress, and energetic pathways.

**Keywords** Myocardial remodelling · Left ventricle reverse remodelling · Energetic deficit · Mitochondria · Reactive oxygen species · Metabolism

## Introduction

The main function of the heart is to provide oxygen and metabolic substrates to all organs, tissues, and cells. Heart failure (HF) occurs when the heart cannot pump enough blood to meet the body's metabolic demands or does it at the expense of high filling pressures [1, 2]. Cardiovascular diseases are the leading cause of death worldwide. In fact, in developed countries, the prevalence of HF is continuously raising [3] at an alarming rate of 1% by year, especially among the oldest and sedentary population [4].

HF results from structural or functional abnormalities in the right or left ventricle (LV) that impairs its filling (diastolic dysfunction, DD) or ejection capacity (systolic dysfunction). These alterations can be asymptomatic for a

long time before the onset of the first HF manifestations [5]. Despite its clinical relevance, the presence of nonspecific symptoms of HF hinders its correct diagnosis and treatment [5].

HF is classically divided in HF with preserved or reduced ejection fraction (EF), HFpEF or HFrEF, respectively [5]. Among all HF cases, those that most concern the medical and scientific community are HFpEF patients. As HFpEF is associated with aging, sedentarism, and obesity and considering the increase of life expectancy and the behaviours of the population, it is presumed that, in 2020, HFpEF will affect more than 8% of the population over 65 years and will represent nearly 69% of all HF cases. Its increased prevalence follows other epidemiologic and social burdens typical of a sedentary lifestyle, such as hypertension, chronic kidney disease [6], type 2 diabetes, anaemia, chronic obstructive pulmonary disease, and atrial fibrillation [7]. Besides representing more than 50% of all HF cases, the higher mortality and

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mainly chronic morbidity impose significant costs to the healthcare system, mostly due to the high frequency of re-hospitalizations [6].

The HFpEF diagnosis remains challenging, mainly in its early stage (Fig. 1). In the ambiguous cases, the evaluation of aerobic capacity determined by echocardiography, performed through a diastolic stress test and using a dynamic exercise protocol, reveals effort intolerance in those patients, an important hallmark of HFpEF [5].

Regarding biomarkers, additionally to the routinely use of BNP, other molecules such as procollagen, interleukin-6 and -8, thyroid hormones, troponin T, proteins involved in the transport of fatty acids (FA), and carbohydrate antigen 125 (CA125) are potential candidates for HFpEF diagnosis, however, still await validation [9]. Recently, the index of some biomarkers ((C-reactive protein + growth differentiation factor-15 + soluble source of tumorigenicity 2)/NT-proBNP) was reported to discriminate HFpEF from HFrEF efficiently [10].

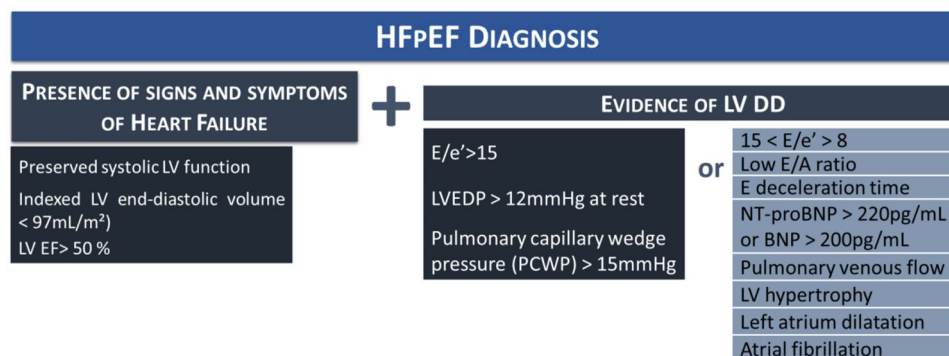
Although initially thought to be less threatening than HFrEF, a prospective population study observed that HFpEF patients present a survival rate similar to those with reduced EF [7]. The limited knowledge about LV remodelling and reverse remodelling (RR) in HFpEF is reflected in the few therapeutic options available to these patients. Regardless of the continuous pursuit to discover novel therapeutic targets, most of them have not proven efficacy in HFpEF. Moreover, some clinical trials have provided disappointing even harmful results, before reaching the phase III [11, 12]. The complexity of HFpEF has due to the multiple cardiac and non-cardiac associated pathologies. To unravel the mechanism behind HFpEF and to understand this syndrome, it is essential to simplify it. The categorization of HFpEF by its specific triggers and phenotypes and their subsequent study

may help to clarify the mechanism behind HFpEF and boost significantly the hope for a new effective treatment.

## Cardiac remodelling and reverse remodelling

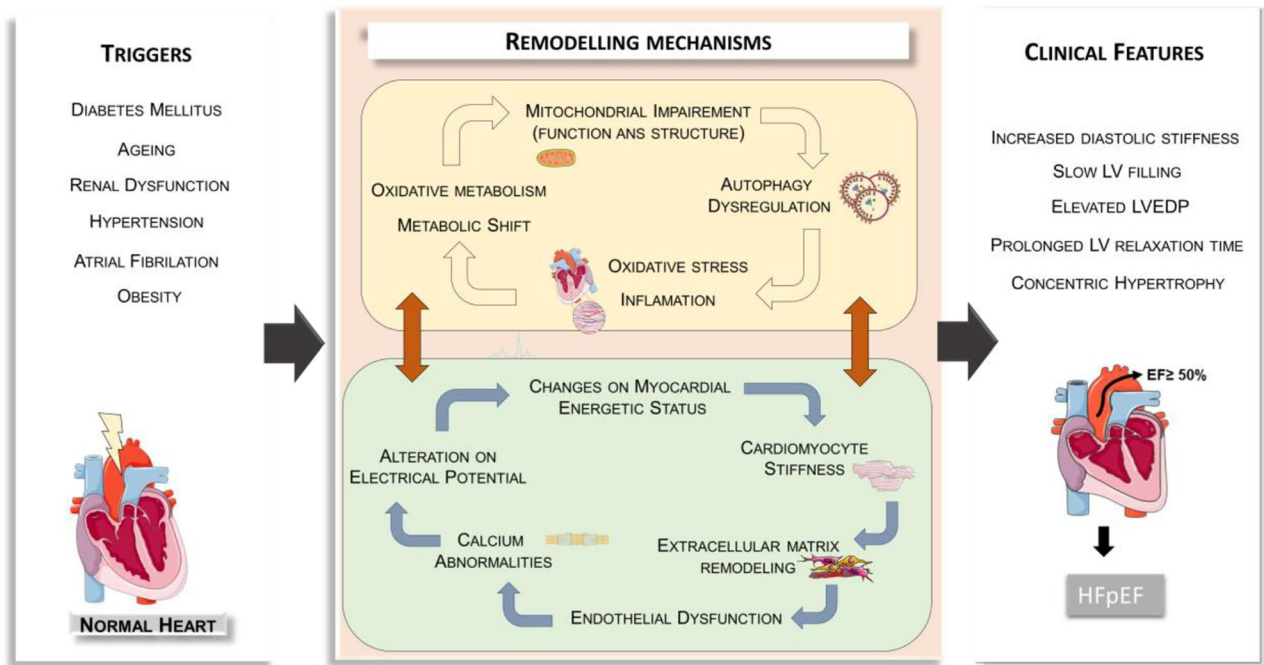
Myocardial remodelling occurs after any stressful stimulus that triggers molecular, cellular, and interstitial changes in the heart. Myocardial remodelling is clinically manifest by alterations in the size, mass, geometry, and function of the heart and occurs in both HFpEF and HFrEF. In contrast, RR is defined as the attenuation or normalization of these cardiac abnormalities [13, 14]. Therefore, to dissect the molecular basis underlying both myocardial remodelling and RR will surely provide a step forwards towards the understanding of HFpEF pathophysiology and its treatment, respectively.

In HFpEF the process of myocardium remodelling induced by different triggers involves abnormalities in energy metabolism, inflammation, and oxidative stress that trigger (or are triggered) by coronary microvascular endothelial dysfunction, infiltration of activated macrophages, and reactive interstitial fibrosis [15]. As a result, hypertrophic and profibrotic signalling cascades become activated, altering the function or expression of myofibrillary and calcium-handling proteins, as well as inducing fibroblast proliferation and an imbalance of proteins that regulate collagen turnover. These cascades of pathological events end with stiffening of the myocardium and abnormalities in the excitation–contraction coupling (ECC) affecting mainly myocyte relaxation, and thus, decreasing ventricular filling and elevating ventricular pressures during diastole, inducing DD, a hallmark of HFpEF (Fig. 2) [16–19].



**Fig. 1** Workflow to diagnose HFpEF. In addition to the imperative presence of signs and symptoms of heart failure, the patients should present preserved left ventricular systolic function and evidence of diastolic dysfunction (DD). DD is determined by pulmonary capillary wedge pressure (PCWP) > 15 mmHg, left ventricular end-diastolic pressure (LVEDP) > 12 mmHg at rest or  $E/e'$  > 15. When  $E/e'$  is in

the range of 8–15 or NT-pro natriuretic peptide type B (BNP)/BNP levels are higher (NT-proBNP > 220 pg/mL or BNP > 200 pg/mL) is necessary at least one additional sign of DD, including a low E/A ratio combined with a high deceleration time, pulmonary venous flow patterns indicative of DD, increase left atrium, atrial fibrillation, and/or LV hypertrophy [8]



**Fig. 2** Mechanisms underlying cardiac remodelling in HFpEF. The figure shows the main triggers of myocardial remodelling and some underlying mechanism responsible for HFpEF-clinical features. EF

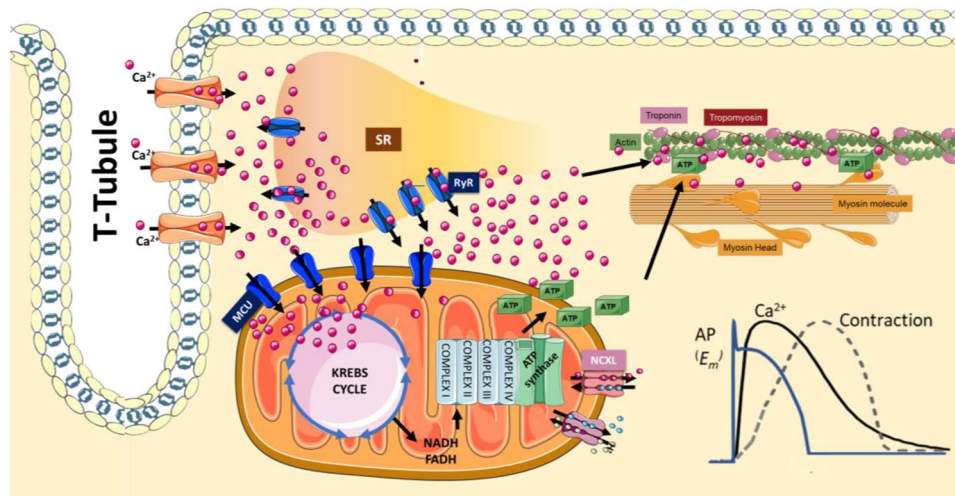
ejection fraction, LV left ventricle, LVEDP left ventricular end diastolic pressure, SR sarcoplasmic reticulum

The most recent assumptions in the aetiology behind HFpEF are based on the augment of systemic inflammation and metabolic disarrangement, both triggered by HFpEF non-cardiac comorbidities [18, 20]. The shift in the preferred oxidative substrate, the increase of reactive oxygen species (ROS), and mitochondria dysfunction causes energy depletion due to a mismatch between ATP production and demand. Concomitantly several downstream signalling pathways are activated, disrupting the normal cardiac architecture, triggered systemic inflammation that can induce endothelial dysfunction of the coronary vasculature and other deleterious processes. Together these mechanisms drive the heart to remodel and stiffen, while in HFrEF prevails the cardiomyocyte loss mainly due to ischemia and the heart muscle becomes weak, thin, or floppy [21, 22].

It has been previously suggested that abnormal myocardial mitochondrial energetics and oxidative stress precede structural remodelling [23]. In fact, all the mechanisms involved in cardiac remodelling may be potentially associated with mitochondrial dysfunction, although this has not been extensively addressed yet. Indeed, only a few studies have reported metabolic disturbances in experimental models of myocardial hypertrophy and DD [24] and in HFpEF patients [25] and no therapeutic approach has yet been directed towards the mitochondria.

### Myocardial excitation–contraction coupling and cytosolic-mitochondria calcium handling

Cardiac ECC is an orchestrated process only possible due to the conversion of an electrical stimulus into mechanical force (excitation to contraction) [26]. ECC begins with myocyte electrical excitation that triggers  $\text{Ca}^{2+}$  entry, its release from the sarcoplasmic reticulum (SR), and subsequent myofilament activation/contraction with sarcomere shortening (Fig. 3) [27]. The mitochondria also participate in this process as it uptakes cytosolic  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_c$ ) and uses it to regulate energy metabolism, including ATP regeneration. Thus, changes in mitochondria are expected to impact myocardial contraction and relaxation and vice versa [28]. This organelle owns all the molecular machinery for efficient  $\text{Ca}^{2+}$  cycling (uptake and extraction) that should work in a well-coordinated fashion to match any change in energy demand in a process named excitation-energetic coupling. Some studies suggested that mitochondrial  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_m$ )-cycling in adult ventricular myocytes is coordinated with ECC in a beat-to-beat basis, meaning that mitochondrial  $\text{Ca}^{2+}$  uptake oscillates in response to  $\text{Ca}^{2+}$  transients and are pacing-dependent (Fig. 3) [29, 30]. At the molecular level, mitochondrial



**Fig. 3** Myocardial excitation–contraction and excitation-energetic couplings. After reaching the T-tubules, the action potential induces  $\text{Ca}^{2+}$ -channel opening and allows the  $\text{Ca}^{2+}$  inflow to the junctional zone or dyadic cleft. This  $\text{Ca}^{2+}$  wave triggers ryanodine receptor (RyR) opening and abundant  $\text{Ca}^{2+}$  release ( $10^{-7}$  to  $10^{-5}$  M) from the sarcoplasmic reticulum (SR)—calcium-induce-calcium release. SR is the major intracellular  $\text{Ca}^{2+}$ -storage organelle into the cell that is located closely to T-tubules to facilitate the ECC [26, 37]. Sarcoplasmic  $\text{Ca}^{2+}$  binds to TnC and induces a conformational change in TnC that exposes the binding site of myosin ATPase located on the myosin head on the actin molecule. This process requires ATP hydrolysis to supply energy for the conformational change that occurs in the actin-myosin complex. Thus, when the amplitude and/or frequency of the  $\text{Ca}^{2+}$  transient rises, cardiac work increases, and therefore, both

$\text{Ca}^{2+}$  cycling and ATP hydrolysis augmented  $[\text{Ca}^{2+}]_m$  rises rapidly and with similar velocities as cytosolic  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_c$ ), allowing for fast adjustment of mitochondrial ATP production to the increased myocardial demands—excitation-energetic coupling. At the matrix,  $\text{Ca}^{2+}$ -stimulates Krebs cycle enzymes (pyruvate-,  $\alpha$ -ketoglutarate, and isocitrate dehydrogenases) to regenerate NADH and  $\text{FADH}_2$  but also complex III of the ETC and the ATP synthase to accelerate respiration. As a result of these coordinated processes, ATP and  $\text{Ca}^{2+}$  allow the sliding movement between the myosin heads and the actin myofilaments as long as the cytosolic  $\text{Ca}^{2+}$  remains elevated (plateau phase) and leading to sarcomere shortening. ATP, adenosine triphosphate; DHP, dihydropyridine receptors; ETC, electron transport chain; RyR, ryanodine receptors; SR, sarcoplasmic reticulum; TnC, troponin C; and TnI, troponin I

$\text{Ca}^{2+}$  influx (MCU) and mitochondrial lithium/sodium-calcium exchanger (NCLX) controls  $\text{Ca}^{2+}$  influx and efflux, respectively, thus representing key players in coordinating  $[\text{Ca}^{2+}]_c$  and  $[\text{Ca}^{2+}]_m$  [31]. Besides MCLX,  $\text{Ca}^{2+}$  release from the mitochondria also depends on the mitochondrial calcium buffering capacity. For higher heart rates, the kinetics of  $\text{Ca}^{2+}$  decay is slowest in mitochondria than in cytosol, contributing to calcium accumulation in mitochondria, presumably to stimulate Krebs cycle dehydrogenases. However, when in excess,  $[\text{Ca}^{2+}]_m$  can deleteriously activate apoptosis pathways [32]. The susceptibility to  $(\text{Ca}^{2+})$ -induced changes on mitochondrial permeability transition pore (an early indicator of mitochondrial dysfunction) has been described to be increase in a pig model of HFpEF [33].

While during diastole cytosolic and mitochondrial  $[\text{Ca}^{2+}]$  are similar, after stimulation  $[\text{Ca}^{2+}]_m$  easily reach values 10- to 100-fold higher than in the cytoplasm [34, 35]. The higher rate of mitochondrial  $\text{Ca}^{2+}$  uptake in response to relatively small increases in  $[\text{Ca}^{2+}]_c$  contrasts with the lower MCU  $\text{Ca}^{2+}$  affinity ( $K_d$  about 20–50  $\mu\text{M}$ ), [35]. To overcome lower  $K_d$ , it is mandatory the presence of an upper physiological  $[\text{Ca}^{2+}]_c$  range that can transiently be reached at microdomains of high  $[\text{Ca}^{2+}]$  named as  $\text{Ca}^{2+}$  hot spots (Fig. 3). At

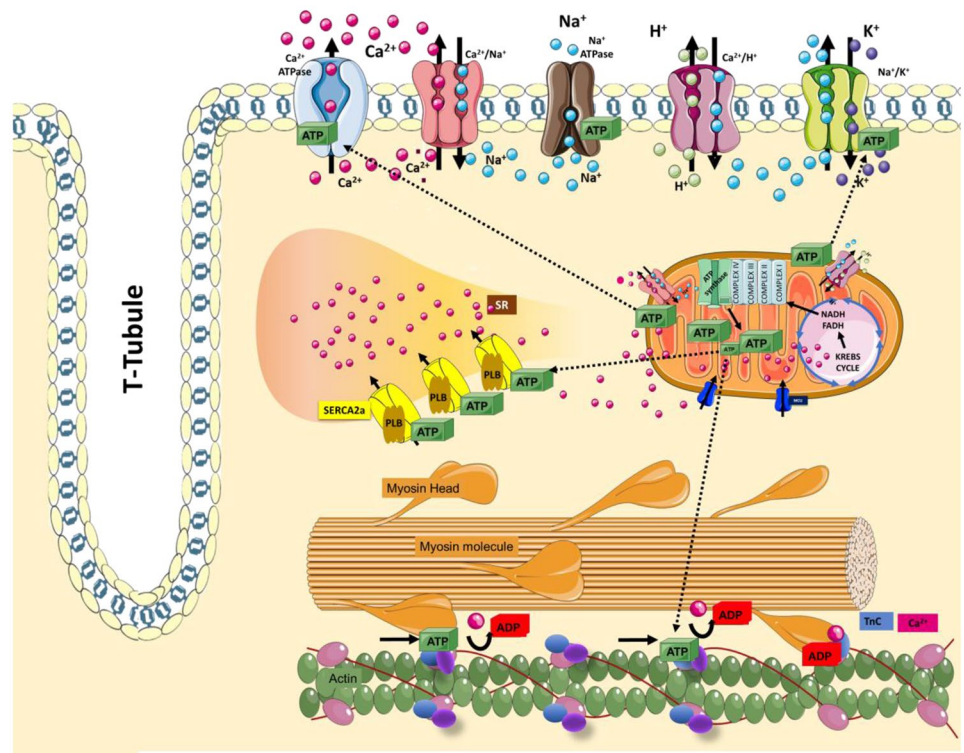
these locations mitochondria are strategically located close to  $\text{Ca}^{2+}$  channels such as RyR or SR channels [35, 36]. Furthermore, MCU and NCLX are spatially separated in cardiac mitochondria. Mitochondria that contact with SR at the zones of  $\text{Ca}^{2+}$  hot spots are rich in MCU but lack NCLX. This distribution minimizes the energy cost of maintaining mitochondrial  $\text{Ca}^{2+}$  signals [28, 31].

For cardiac relaxation to occur the amount of intracellular  $\text{Ca}^{2+}$  must decline, which requires inactivation of L-type  $\text{Ca}^{2+}$  channels and sequestration of  $\text{Ca}^{2+}$  by the activation of (1) sarcoplasmic reticulum calcium-ATPase (SERCA), which pumps  $\text{Ca}^{2+}$  back into the SR; (2) sarcolemmal  $\text{Na}^+/\text{Ca}^{2+}$  exchange (NCX); (3) sarcolemmal  $\text{Ca}^{2+}$ -ATPase which pumps  $\text{Ca}^{2+}$  directly out of the cell; and (4) mitochondrial  $\text{Ca}^{2+}$  uniport which pumps  $\text{Ca}^{2+}$  into the mitochondria [38–40].

In the presence of a normal  $[\text{ATP}/\text{ADP}]$ , as cytosolic  $\text{Ca}^{2+}$  decreases,  $\text{Ca}^{2+}$  dissociate from TnC, induces a conformational change on TnI back to its original shape and the restoration of tropomyosin inhibition of actin-myosin interaction, preventing the formation of cross-bridges during relaxation [41]. The cycle ends with the binding of a new ATP to the myosin head, displacing the ADP and restoring the initial sarcomere length (Fig. 4).



**Fig. 4** Myocardial requirements of ATP during diastole. During relaxation, ATP hydrolysis is necessary to release ADP from myosin heads and uncouple myosin heads from actin filaments, dissociating  $\text{Ca}^{2+}$  from troponin C, to reuptake  $\text{Ca}^{2+}$  to SR and to remove  $\text{Ca}^{2+}$  out to the cytoplasm. ATP adenosine triphosphate, PLB phospholamban, SR sarcoplasmic reticulum, SERCA2a sarco/endoplasmic reticulum calcium-ATPase pump, TnC troponin C



Relaxation is a key player of HFpEF pathophysiology; thus, it is not surprising that modifications in proteins that mediated  $\text{Ca}^{2+}$  cycling, such as NCX, SERCA, PLB and RyR2, have been reported during cardiac remodelling in HFpEF or compensated hypertrophy [42]. In cardiomyocytes from an HFpEF rat model with chronic kidney disease or pigs with metabolic syndrome, cell shortening amplitude,  $\text{Ca}^{2+}$  transients, and kinetics of shortening were altered [33, 43, 44]. Also, at higher workloads, isolated hearts from rats with metabolic syndrome and diabetes have shown incapacity to upregulate ATP synthesis and decreased free energy of ATP hydrolysis ( $\Delta G_{\sim\text{ATP}}$ ), which affects diastolic relaxation and impaired contractile reserve [45, 46].

### HFpEF: a metabolic disorder?

An increasing body of evidence supports that HF is a metabolic disease that presents abnormalities in mitochondrial oxidative metabolism and dysregulation of fatty acid (FA) and glucose transport/utilization. Altogether these abnormalities lead to an energy deficit. Nevertheless, the heart is an organ extremely flexible, capable of generating energy from the oxidation of several substrates, such as fatty acids, carbohydrates (lactate, glucose), ketones, and amino acids [47]. Thus, targeting alterations in cardiac metabolism seems a reasonable approach to increase cardiac efficiency. However, the use of metabolic modulators is reliant on our

understanding of the complex changes occurring in cardiac energy metabolism in the healthy and failing heart that depend not only on the HF- type and severity but also on the co-existence of other co-morbidities [48]. Furthermore, among the vast available experimental studies, this complexity is further exacerbated by differences in the animal strain and study design [13, 14] or by the different triggers of HF [48].

For instance, the use of animal models with distinct cardiovascular risk factors (such as type I and type II diabetes, obesity, hypertension), the distinct evaluation time-points or even the use of distinct regions of cardiac tissue to perform molecular, cellular, or histological studies can introduce mixed results and overshadow the general conclusions. Hence, before reviewing the metabolic alteration in HF and HFpEF, we will briefly recall carbohydrates and FA metabolism.

### Physiology of myocardial energy metabolism

The cardiomyocytes' plasticity is essential for the myocardium to meet its high metabolic demands and to quickly adapt to the constant hemodynamic and/or metabolic challenges [49]. Compared with other cells, cardiac myocytes are those who consume more energy. Under normal conditions approximately 70 to 90% of cardiac ATP comes mainly from mitochondrial oxidation of FA and the remaining 10

to 30% is provided by carbohydrates (glucose and lactate) and ketones [48, 50]. This scenario changes under disease conditions like HF, cardiac hypertrophy, HFpEF, and ischemia, wherein a metabolic transformation occurs as the heart becomes exposed to increased haemodynamic load and oxygen tension [51, 52].

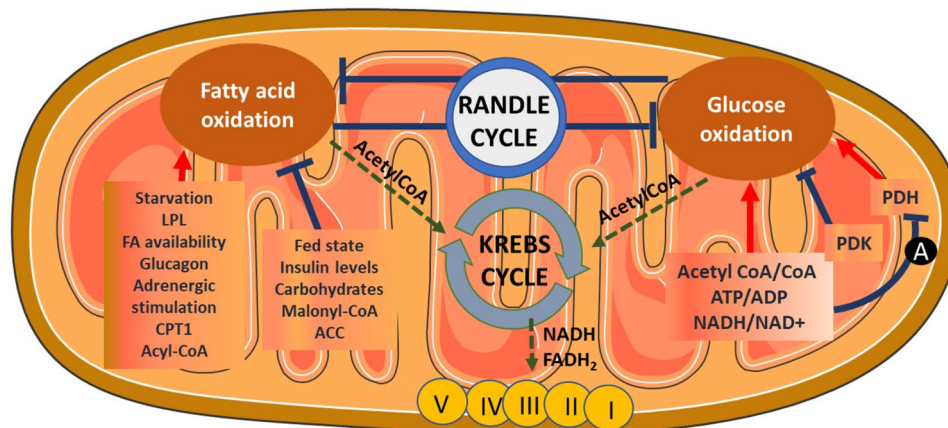
### General consideration—carbohydrate and lipids metabolism

Glucose transporter-4 (GLUT4) and -1 (GLUT1) mediate the glucose uptake into the cells. Glycolysis is the first step of carbohydrate metabolism and converts glucose into pyruvate in the cytosol. Pyruvate is then translocated inside the mitochondria by pyruvate translocase, and under aerobic conditions, is converted to acetyl-CoA by the pyruvate dehydrogenase complex during glucose oxidation. Pyruvate dehydrogenase (PDH) is allosterically activated and negatively regulated by pyruvate dehydrogenase kinase (PDK) and by increased levels of acetyl CoA/CoA, ATP/ADP, and NADH/NAD<sup>+</sup>. In contrast, PDK is positively regulated by pyruvate dehydrogenase phosphatase (PDP), which is activated by reduced levels of these ratios [53].

In the mitochondrial matrix, acetyl-CoA enters the tricarboxylic cycle (TCA) to generate NADH from NAD<sup>+</sup>, which act as an electron donor to complex I of the electron transport chain. Under hypoxia, anaerobic glycolysis is promoted by HIF1-mediated expression of PDK and glycolytic enzymes [54].

Free FAs released from adipose tissue in the fasted state by hormone sensitive lipase (HSL) or FA from lipoprotein-associated triacylglycerols via the action of lipoprotein lipase (LPL) are released into the plasma and reach the heart to be uptake by cardiomyocytes.

Lipolysis is regulated by catecholamines, glucocorticoids, glucagon, and insulin that controls LPL and HSL activity. Upon feeding, insulin activates LPL while inhibits HSL, promoting the uptake of free FA from the blood. Contrarily, during fasting, diabetes, intense exercise, or stress conditions, glucagon activates HSL, promoting fat tissue lipolysis [55]. In HF, the low cardiac output can be compensated by a hyperadrenergic state that increases plasmatic catecholamines increasing plasmatic free FA concentration. As higher levels of free FA inhibit glucose utilization, increased plasma catecholamines are typically associated with insulin resistance [56]. FA uptake to cardiomyocytes can occur by passive diffusion or mediated by several proteins including FA translocase (FAT/CD36), FA-binding protein (FABP), and FA transport protein (FATP) [57]. Within the cardiomyocyte, the enzyme fatty acyl-CoA synthase will convert long-chain FA to fatty acyl-CoA. Fatty acyl-CoA are converted to acyl-L-carnitine and transported into the mitochondria by the enzymes carnitine palmitoyl transferase type 1 (CPT-1) and CPT-2 (localized in the outer and inner mitochondrial membrane, respectively), through a process called ‘carnitine shuttle’. Afterwards, acyl-L-carnitine is converted back to long-chain acyl-CoA FA and undergoes  $\beta$ -oxidation, producing acetyl-CoA used



**Fig. 5** Regulation of fatty acid and glucose oxidation in the mitochondria. The oxidation of metabolic substrates, namely, fatty acid, and glucose are tight regulated. The red arrows represent some mechanisms involved in the activation of FA and glucose oxidation. Blue arrows indicate some mechanisms involved in the inhibition of these metabolic processes. High levels of acetyl CoA/CoA, ATP/ADP, and NADH/NAD<sup>+</sup> inhibit PDH. The main purpose of fatty acid and glucose oxidation are to produce Acetyl CoA (green arrows) that will enter Krebs cycle to produce NADH and FADH<sub>2</sub>, electron donors to

complex I and II of electron transport chain, located at inner mitochondrial membrane. The Randle cycle or the glucose/FA cycle represents the feedback inhibition of FA and glucose oxidation mediated by metabolic intermediates. ACC, acetyl-CoA carboxylase; ADP; adenosine di-phosphate; ATP, adenosine triphosphate; FA, fatty acid; CPT1, carnitine-palmitoyl transferase-1; PDH, Pyruvate dehydrogenase; PDP, dehydrogenase phosphatase; PDK, pyruvate dehydrogenase kinase; and NAD<sup>+</sup> /NADH, nicotinamide adenine dinucleotide oxidized and reduced

in TCA cycle to generate NADH and FADH<sub>2</sub> providing reducing equivalents for the electron transport chain.

FA and glucose oxidation have multiple and tight regulation sites, which are essential to maintain the heart ability to switch between available substrates (Fig. 5) [58].

The regulation of FA oxidation starts with its uptake to the mitochondria by CPT1. CPT1 activity decreases in the fed state, reducing FA oxidation. During fasting, FA oxidation and CPT1 activity increase. The initial intermediate of FA biosynthesis, Malonyl-CoA, is a potent CPT1 inhibitor. Under starvation ACC is inhibited by acyl-CoA (decreasing malonyl CoA) and the CPT1 inhibition is released allowing more acyl-CoA to be oxidized.

Glucose oxidation is controlled by pyruvate dehydrogenase complex. PDH is allosterically activated and negatively regulated by PDK and by upregulation of acetyl CoA/CoA, ATP/ADP, and NADH/NAD<sup>+</sup>. In contrast, PDK is positively regulated by pyruvate PDP, which is activated by downregulation of these ratios. In the fed state PDH is active and becomes inactive under starvation (Fig. 5).

Furthermore, the Randle cycle (also called Glucose–fatty acid cycle), is a biochemical mechanism, whereby glucose and FA compete for oxidation and uptake. The cycle controls fuel selection and adapts the substrate supply and demand. As an example, in the muscle, when FA oxidation is intense, a significant reduction in the uptake and utilization of glucose takes place [48].

### Cardiac metabolism in HF

When exposed to sustained pressure overload, other hemodynamic stress or changes in myocardial blood flow, the heart remodels first metabolically and then structurally [59]. Indeed, it is widely accepted that under these stimuli, the failing heart reverses back to a foetal phenotype possibly to protect the failing heart from further irreversible structural and functional impairment [51, 60]. However, the exact energetic metabolic profile in HF remains controversial as denoted by the discrepant results on literature regarding substrate preferences. While sometimes the heart switches from FA oxidation to glucose metabolism, under other circumstances as HF associated with obesity or diabetes, the heart prefers to oxidized FA {Karwi, 2018 #384}. Furthermore, some clinical and experimental studies suggest that FA oxidation is either not changed or is increased in HF [51, 61, 62]. The complexity of the theme is also reflected on the diversity of the therapeutic options that have been proposed (supplement 1).

The benefit of glucose utilization compared with FA is a lower O<sub>2</sub> consumption rate [63]. Each glucose consumes 6 molecules of O<sub>2</sub> with an ATP yield of 31 and a P/O ratio of 2.58. Inversely, the oxidation of FA palmitate consumes 23 molecules of O<sub>2</sub> with an ATP yield of 105, giving a P/O

ratio of 2.33, making FA the least efficient energy substrate to the myocardium. Furthermore, FA efficiency is decreased given the requirement of 2 ATP molecules hydrolysis for the esterification of FA to fatty acyl CoA and for the cytoplasmic cycling of FA between fatty acyl CoA and TG and back to FA [48]. This would only represent an advantage assuming the high demand of ATP by the myocardium and the limited ATP storage that can only assure a few seconds of beating [64].

However, the preferential use of glucose as energetic substrate would represent an advantage only if the oxygen availability and the mitochondrial oxidation capacity of the failing heart were preserved to allow glucose oxidation [47, 48], which does not seem to be the case. In fact, transcriptional regulation and expression of hypoxia and mitochondrial oxidative metabolism genes, including glucose oxidation, are decreased in HF, rendering the heart more reliant on glycolysis as a source of ATP regeneration [60, 65].

These phenomena can occur due to the limited capacity of the failing heart to channel the higher amount of uptake glucose into the glycolytic/oxidative pathway with consequent re-direction towards the pentose phosphate pathway. This metabolic pathway is known to increase oxidative stress and induces ‘myocardial glucotoxicity’ by enhancing NADPH levels and thus, superoxide generation by NADPH oxidase [66]. The pentose phosphate pathway was found to be activated in Dahl salt-sensitive rats with congestive HF [65]. Glucose 6 phosphate dehydrogenase (G6P) (a rate-limiting intermediate of pentose phosphate pathway) is known to be positively regulated by insulin, mammalian target of rapamycin (mTOR), AKT, among others [67]. Furthermore, in heart perfused with glucose and working at high load, the augment of G6P activates insulin-dependent mTOR signalling, impairing cardiac power and inducing ER stress response. In human HF mechanical unloading decreases levels of G6P, reduces mTOR activation, and relieves ER stress [59].

### Cardiac glucose metabolism in hypertrophy, DD and HFpEF

Unlike physiological hypertrophy, where an increase of FA and glucose oxidation is observed, pressure overload-induced hypertrophy switches main myocardial source for oxidative energy from FA to glucose [68–73] with downregulation or no changed of FA metabolism genes [68–70, 74].

The uncoupling of glycolysis from glucose oxidation decreases cardiac efficiency and contributes to the energy deficit as well as to the severity of hypertrophy and HF secondary to pressure-overload [75]. In HFpEF (induced by metabolic and/or hemodynamic stress) the overall decrease in mitochondrial oxidative metabolism can result in a compensatory rise in glycolysis rate, increasing the uncoupling between glycolysis and glucose oxidation [60, 76].



Intriguingly, insulin resistance was associated with improved contractile function maybe by protecting the hearts from fuel overload, reducing glucose uptake and increased rates of glucose oxidation [76, 77]. Likewise, in hypertensive patients an inverse correlation between the rate of glucose uptake and the presence and severity of DD was found [73].

The underlying reason for the deleterious effect of glucose oxidative metabolism uncoupling is not fully understood. In addition to the deviation towards pentose phosphate pathway and the augment of oxidative stress, the excessive production of lactate and protons decreases pH and can reduce the  $\text{Ca}^{2+}$  sensitivity on troponin C, inhibit the slow  $\text{Ca}^{2+}$  current, and decrease the myocardium contractility, contributing to the development of HFpEF [60]. Furthermore, the additional need for ATP to remove these protons and maintain sodium and calcium homeostasis decreases cardiac efficiency and contributes to the deterioration in cardiac function [78][60]. Similarity to proliferative cancer cells, the ‘Warburg effect’, characterized by higher glycolysis rates even under aerobic conditions and by an overall decrease in mitochondrial oxidative metabolism, is likely to occur under conditions of cardiac hypertrophy and DD, contributing to cardiac dysfunction and its subsequent progression to HFpEF [60, 69, 79, 80].

### Cardiac FA metabolism in hypertrophy, DD, and HFpEF

The evidence of reduced FA oxidation in cardiac hypertrophy derives from studies in humans and animals with genetically or chemically impairment of FA oxidative capacity [81]. Recently, Hunter and colleagues described a distinct metabolic signature between HFrEF from HFpEF. Both HFpEF and HFrEF patients showed increased plasma long-chain acylcarnitines, further augmented in HFrEF, highlighting a more severe impairment in mitochondrial FA oxidation enzymes or more uncoupled FA oxidation in the latter [82].

A reduction/blockade in FA oxidation has been shown to promote DD and hypertrophy by mTOR activation [81], while maintenance of FA oxidation by overexpressing Acetyl Coenzyme A Carboxylase attenuates hypertrophy, reduced fibrosis, oxidative stress, and preserved mitochondrial function in rats with DD induced by AngII [83]. Additional evidence of the benefits of improving FA oxidation comes from HFpEF mice submitted to pressure overload and hypertrophic myocardial cells where the stimulation of  $\beta$ -oxidation by PPAR $\alpha$  with Astragaloside IV improved mitochondrial function, ATP content, and increased SERCA2a [84]. In a metabolic rat model with insulin resistance, the poor myocardial recovery pattern after ischaemia–reperfusion resulted from the inability to increase or sustain long-chain FA oleate oxidation. When oleate was replaced by a medium-chain FA (more permeability to enter the mitochondria), contractile function improved [85]. However, as observed in metabolic

heart disease and diabetes, the loss of metabolic flexibility favouring FA oxidation can also be detrimental to cardiac function [18]. Alterations in the oxidative substrate due to mutations in metabolic pathways or an imbalance between FA uptake and oxidation can result in myocardial energy deficiency, lack of crucial membrane components (e.g., cardiolipin deficiency), excessive lipid accumulation and lipotoxicity, ultimately leading to cardiac hypertrophy, dysfunction, or other associated cardiac pathologies [86]. Thus, metabolic modulation seems to have a profound impact in cardiac diseases changing contractile function and contributing to HF progression. Nevertheless, a deeper understanding of these complexes pathways urges and might pave the way for better management of HFpEF.

### Ketone metabolisms in health and HF conditions

Ketogenesis is an alternative biochemical process to produce ketone bodies through breakdown of FA and ketogenic amino acids. During fasting, starvation, or periods of high-intensity exercise training, ketone bodies are produced in the liver by the incomplete oxidation of free FA released from adipose tissue and used as an alternative source of energy. The resultant metabolites are acetone, excreted in the breath,  $\beta$ -hydroxybutyrate ( $\beta$ OHB), and acetoacetate, both entering blood circulation [61]. The use of ketone by the heart has long been described and could represent ~ 2.6% ( $\beta$ OHB) and ~ 6.0% (acetoacetate) of total metabolism oxidation in the heart.

As presumably the heart itself cannot do ketogenesis, it uptakes ketonic bodies to cardiomyocytes and transports them to the mitochondrial matrix where  $\beta$ OHB is oxidized into acetoacetate by  $\beta$ OHB dehydrogenase (BDH1) [87]. Thereafter, the rate-limiting enzyme, succinyl-CoA:3-oxoacid-CoA-transferase (SCOT), transforms acetoacetate into acetoacetyl-CoA that is lastly converted to acetyl-CoA by acetyl-CoA acetyltransferase, acetyl-CoA goes in the TCA cycle. The regulation of ketone metabolism in the heart is poorly understood although the level of ketone bodies in the blood is a key determinant of its oxidation rates. Furthermore, hepatic BDH1 has been shown to be post-translationally modified by SIRT5 and cardiac SCOT may be regulated by residue-specific nitration in the settings of diabetes and aging [61].

When the overall oxidative pathways are impaired the heart relies more on ketone bodies to regenerate ATP as repeatedly demonstrated in humans and in experimental studies [88–90]. In mice submitted to chronic pressure overload, ketone metabolism is an important player for the development of cardiac remodelling. SCOT knockout mice denoted increase of oxidative stress, mitochondria, and myofibrillar disarrangement as well as a worsening of cardiac function [91].



Recently but supporting old published results was demonstrated an upregulation of ketone body synthesis enzymes' in diabetic rats. These results suggest that the heart is capable to synthesize ketone bodies when mitochondrial fatty acid oxidation is excessive. This metabolic pathway may represent a mechanism to fight 'metabolic inflexibility' and improve glucose oxidation through the pyruvate dehydrogenase complex, by deviate acetyl-CoA derived from elevated FA oxidation into ketone bodies, decreasing acetyl-CoA/CoA ratio [92].

Recently, ketone bodies have been gaining attention due to the striking and unexpected results obtained in the EMPAREG OUTCOME trial. This clinical trial used an inhibitor of sodium-glucose cotransporter 2 (SGLT2), empagliflozin, in patients with type 2 diabetes and high cardiovascular risk [93]. In addition to enhancing diuresis and reducing blood pressure, empagliflozin induced a mild and persistent hyperketonemia allowing  $\beta$ OHB to be taken up by the heart (among other organs) and oxidized in preference to FA. By improving oxygen consumption at mitochondria (the P/O ratio of  $\beta$ OHB, is 2.50, less efficient than glucose but more efficient than palmitate) [61] and due to the elevation of haematocrit, this alternative metabolic substrate improved tissue oxygenation, thereby establishing a powerful synergy with the metabolic substrate shift [94]. Moreover, ketone bodies decreased circulating FA by inducing its uptake into adipocytes while stimulating glucose uptake into myocytes, improving substrate supply and possible energy production in the heart [18]. Until now, it remains obscure if the higher ketone body metabolism in HF is a cause, a consequence, a bystander, or a compensating mechanism, mostly because its cardiac metabolism and its effect under disease conditions are poorly understood [18].

### Energetic deficit in HFpEF: looking deep into the mitochondria

Many of the comorbidities associated to DD and HFpEF are related to cardiac metabolic perturbations capable of modifying the amount of adenine and creatine compounds, and thus affecting myocardial function [95]. Actually, a linear relationship has been described between ATP hydrolysis, actin-myosin interaction, and contractile force [3, 96–98] as well as between ADP levels and end-diastolic pressures [99].

In HFpEF, the evidence of an energy deficit is confirmed by reduced PCr/ATP due to PCr decrease [100] or, in some cases, to a drop of 30–40% in ATP levels in failing hearts. In pathological hypertrophy, the increase of glycolysis activity [74] culminates in a decrease of mitochondrial oxidative metabolism and thus a reduction in the content of ATP, Cr, and PCr [101, 102]. In fact, the reduction of PCr levels results in a deficit of conversion of ADP to ATP and impairs

the ratio ATP/ADP [99]. Moreover, elevated levels of ADP augment the myofilaments  $\text{Ca}^{2+}$  sensitivity and increase the recruitment of cross-bridges, culminating in a slower myocardial relaxation [100, 103, 104] and higher cardiomyocytes stiffness [103]. The increase of sarcomere  $\text{Ca}^{2+}$  sensitivity and ATPase utilization results in the augment of energy requirements and compromises the ability of cardiomyocytes to maintain sufficient energy levels resulting in DD [105]. In rats, the reduction of the contractile reserve, the increase of LV end-diastolic pressure, and mortality has been associated with PCr reduction or a creatine kinase (CK) inhibition [99]. In order to prevent ADP accumulation, PCr is used re-phosphorylate ADP at the cytosol while the entry of Cr and ADP to mitochondria stimulates ATP regeneration by accelerating electron flux along the ETC and thus boosting oxidative phosphorylation [28].

In the myocardium, free energy resulting from ATP hydrolysis is used for several mechanisms, being 60–70% directed to contraction and relaxation and the remaining 30–40% used by sarcoplasmic reticulum (SR)  $\text{Ca}^{2+}$ -ATPase (SERCA-2a) and by others ion pumps [3, 106]. A relevant fact for HFpEF is the finding that the diastolic consumption of ATP exceeds the one of systole [106]. Therefore, an abnormal relaxation associated to DD, as occurs in HFpEF, can also result from deficient ATP and ADP cycling, which induce alterations in filaments sensitivity to  $\text{Ca}^{2+}$  and diastolic reuptake of  $\text{Ca}^{2+}$  (Fig. 4) [107, 108]. In HFpEF, alterations on  $\text{Ca}^{2+}$  reuptake could result from the reduction of SERCA-2a activity or an increase in the SERCA-2a inhibitor, phospholamban. Both can change myocardial  $\text{Ca}^{2+}$  kinetics and ventricular relaxation [106, 109, 110]. Additionally, hyperphosphorylation of ryanodine receptors can also underlie a reduction of SR  $\text{Ca}^{2+}$  content (due to  $\text{Ca}^{2+}$  leakage) and therefore contribute to increasing  $\text{Ca}^{2+}$  cytosolic and worsening myocardium relaxation [95, 108]. These pieces of evidence support that normal diastole depends on the proper function of metabolic processes capable of regenerating ATP. Thus, low energy reserve observed in HFpEF can result from mitochondrial dysfunction or metabolic disturbances. However, it remains to clarify if these alterations are the cause or the consequence of HF (Fig. 4)[3].

One cannot describe alterations in energetic metabolism in HFpEF without referring to mitochondria, the powerhouse of the cell. The relationship between mitochondrial dysfunction and HF was suggested in 1962 when Schwartz and Lee observed a reduction in mitochondrial oxidative phosphorylation capacity in pigs that developed HF after aortic constriction [111]. Mitochondrial volume represents over 30% of cardiomyocytes' total volume, reflecting the importance of this organelle and oxidative phosphorylation for the heart. In fact, on adult individuals, under normoxia and resting conditions, about 95% of the myocardial ATP demands comes from

mitochondria [3, 50]. Structural and functional alterations at this organelle have been described in several cardiovascular diseases and in HF. The proposed mechanisms for mitochondrial dysfunction are diverse and include (i) electron transport chain dysfunction, (ii) modifications in mitochondrial complexes organization, (iii) increased oxidative stress, (iv) altered membrane lipid profile with decreased cardiolipin content, (v) changes in the TCA cycle, (vi) mitochondrial uncoupling [112], (vii) decreased transcriptional regulation of mitochondrial biogenesis and FA oxidation, and (viii) altered autophagy and mitophagy [48, 113].

### Mitochondrial subpopulations

In cardiomyocytes, the three mitochondrial subpopulations are distinguished by their subcellular spatial arrangement and morphology: (1) perinuclear mitochondria are located on the nuclear poles and are mostly spherical in shape; (2) subsarcolemmal (SSM) mitochondria are larger, present a lamellar morphology and are situated just below the sarcolemma; and (3) intermyofibrillar (IFM) mitochondria are smaller, have less internal complexity, and are positioned between the myofilaments [114, 115].

This heterogeneity may dictate differences in mitochondrial subpopulations. Indeed, perinuclear mitochondria have been suggested to be more dedicated to generating ATP driving mitochondrial metabolism closer to the nucleus. Moreover, the higher activity rates at complex I, III, IV, and V in IFM than SSM support its primary involvement to supply ATP for contraction, while SSM are more prone to contribute with ATP for active transport across the sarcolemma [116, 117].

In response to deleterious stimuli, such as pressure overload, the pathophysiological response of mitochondrial populations should differ accordingly to its location within the myocyte. Cardiac hypertrophy is more likely to negatively impact IFM compared with SSM as it increases the distance for oxygen and metabolites to diffuse and thus the oxygen tension in the middle of the cell [116, 117]. In a genetic rat strain of type 2 diabetes, SSM subpopulation is structurally and functionally more affected than IFM while in type 1 diabetes is the opposite [118, 119]. In response to ischemia, cardiac SSM are more susceptible to be damaged and to suffer apoptosis caused by  $\text{Ca}^{2+}$  overload-mediated cytochrome C release [120]. The response to oxidative stress also differs among mitochondrial subpopulations. The vulnerability of respiratory chain complexes to post-translational modifications induced by oxidative stress revealed a predisposition of SSM respiratory

chain complexes to carbonylation, whereas IFM respiratory chain complexes were more susceptible to nitration [121].

### Mitochondrial biogenesis and autophagy

Mitochondrial biogenesis can be defined as the growth in size (fusion) or in number (fission) of pre-existing mitochondria mediated by proteins involved in mitochondrial dynamics [122, 123]. Mitochondrial biogenesis is influenced by several physiological or pathological stimuli including caloric restriction, exercise, oxidative stress, cell division, renewal, and differentiation [123]. The involvement of mitochondrial biogenesis in compensated hypertrophy and DD is controversial since changes in the expression of biogenesis proteins, like PGC-1 $\alpha$ , ERR $\alpha$ , and Tfam, are inconsistent [108, 124]. For instance, in humans and dogs with overload-induced hypertrophy or in a pig model of HFpEF, impaired mitochondrial ultrastructure and decreased biogenesis were associated with deficient mitochondrial oxidative function and a faster progression to HF [33, 125–127]. Also, in humans and rodents with HFpEF, the overexpression of proteins involved in biogenesis, mitochondrial fragmentation, and cristae destruction was suggested to be related to mitochondrial damage and mitophagy activation [128], and thus represent a mechanism triggered by mitochondrial loss and injury [129, 130].

Since the discovery of mitochondrial dynamics in 1914 [131], its biological importance has been repeatedly demonstrated even for the heart, where the dysregulation of the mitochondrial dynamics is known to correlate with the pathogenesis of cardiac diseases [132]. Mitochondrial fission and fusion have opposing roles during *in vivo* cardiac mitochondrial quality control [113]. Mitochondrial fusion allows the exchange of material (metabolites, protein, and DNA) between healthy mitochondria [133] while fission facilitate the control of mitochondrial quality by mitophagy, which removes damaged mitochondria through engulfment in lysosomal vesicles [134, 135]. Mitophagy/autophagy is catabolic processes that promote cell survival by destroying defective mitochondria/cells. In mammals, 3 proteins are involved in mitochondrial fusion, mitofusin (Mfn) -1 and -2 fuse the outer mitochondrial membrane, while optic atrophy (OPA)-1 fuses the inner mitochondrial membrane. Mitochondrial fission is regulated by proteins such as dynamin-related protein-1 (DRP1), mitochondrial fission factor (MFF) [62], and fission 1 homologue protein (Fis1) [63] and increases the number and capacity of mitochondria during cell division [136]. These proteins have been found to have other critical functions. Fusion proteins support the inter-organellar exchange of proteins, substrates, and mitochondrial DNA to enhance the stability as well as improves mitochondria  $\text{Ca}^{2+}$ -ER/SR tethering.

Cardiomyocytes with specific deletions of proteins involved in mitochondrial fission develop HF, exhibiting an accumulation of defective mitochondria and increased mitophagy of the entire mitochondria instead of mitophagy restricted to organelles containing the dysfunctional mitochondrial components. In response to pressure overload, myocardial mitophagy increases possibly as an attempt to prevent mitochondrial damage and maintain ATP production [137]. In type 2 DM, the results concerning myocardial autophagy are incongruent [138–140]. Recently, S-nitrosylation has been suggested to inhibit lysosomal enzymes and thus to impair autophagic flux [141], which agrees with the autophagosome accumulation [142] and elucidates the beneficial effects of mTOR inhibition in diabetic mice [143]. At least in type 2 DM, mitochondrial dynamics seem to be time-dependent, explaining the inconsistency of the published results [144].

A balance between fusion and fission is required to sustain a normal mitophagy flux. When in excess, mitophagy causes unnecessary mitochondrial clearance, reducing the number of mitochondria in the heart while its default leads to accumulation of dysfunctional mitochondria. In the latter, as mitochondria cannot be adequately degraded, they continue to cause and disseminate damage, eventually promoting autophagy of the cardiomyocyte [137]. Mitochondrial dynamics (fission and fusion) work together with autophagy as quality control pathways to counteract the processes leading to mitochondrial dysfunction [145]. The balance of mitochondrial dynamics allows for the reorganization and sequestration of damaged mitochondrial components and is the key for healthy crosstalk between the different mitochondrial quality control pathways [133, 145].

### Mitochondrial coupling, oxidative phosphorylation, and reactive oxygen species

Mitochondrial energy production involves the coupling between electron transfer and oxygen uptake through the electron transport chain (ETC) complexes and the phosphorylation of ADP [146]. At the mitochondrial matrix, the Krebs cycle regenerates the electron carriers (NADH and FADH<sub>2</sub>) that will reduce mitochondrial complex I and II at the inner mitochondrial membrane. Subsequently, a series of redox reactions are channelled through five complexes and two carriers (cytochrome C and ubiquinone) of increasing reduction potential toward oxygen (O<sub>2</sub>), the final electron acceptor [39]. There are two electron transport pathways in the ETC: Complex I/III/IV, with NADH as the substrate and complex II/III/IV, with succinic acid as the substrate. The energy released from electron flow is used to couple a proton gradient across the membrane into the inter-membrane space, generating a chemical ( $\Delta\text{pH}$ ) and electrical potential ( $\Delta\Psi\text{m}$ ) across the inner mitochondrial membrane. Together,

$\Delta\text{pH}$  and  $\Delta\Psi\text{m}$  constitute the proton motive that is an essential component of oxidative phosphorylation to regenerate ATP at F1/F0-ATP synthase (complex V) [147]. In the intermembrane space, the mitochondrial CK transforms creatine (shuttled from the cytosol) into phosphocreatine, which functions as a spatial and temporal buffer of energetic phosphates [39]. The dysfunction of oxidative phosphorylation, evidenced by the decrease of mitochondrial respiration rate and consequent decrease of ATP content, is consistently observed in HF [148] but incongruent in cardiac hypertrophy [149]. In experimental animal studies, cardiac hypertrophy and DD was followed by an overall decrease in cardiac mitochondrial metabolism and ATP synthesis [21]. It has been recently demonstrated in an HFpEF animal model an augmentation of functional uncoupling of the respiratory chain and ATP synthesis [33], decreasing the ADP/O ratio and affecting the oxidative phosphorylation efficiency [150, 151].

During oxidative phosphorylation a small amount of proton leakage occur since some protons can return to the mitochondrial matrix independently of the ATPase activity, decreasing the net yield of oxidative phosphorylation [152]. Proton leak can be categorized into basal proton leak and inducible proton leak. In the former, proton leakage relies on the lipid bilayer constitution of the inner mitochondrial membrane and is not regulated. The latter can be precisely regulated by uncoupling proteins (UCP) and adenine nucleotide translocase (ANT) [153].

The UCPs can be regulated positively by ROS and FA or negatively by a purine nucleotides binding (ATP, ADP, GTP, and GDP) [153]. Changes in the UCP's expressions have been found in several types of HF [74, 154] and LV hypertrophy [68, 70]. In an experimental model of chronic pressure overload, LV expression of UCP2 was dependent on HF-progression, being initially decreased and overexpressed later. Interestingly, in the end-stage of HF, a concomitant increase of TNF- $\alpha$  and a decrease of PCr content were also observed. Given these results, the authors suggested that TNF- $\alpha$  could be involved in the upregulation of UCP2, which, in turn, would be responsible for PCr decrease and, therefore, implied in the lower cardiac energy efficiency observed in HF [74].

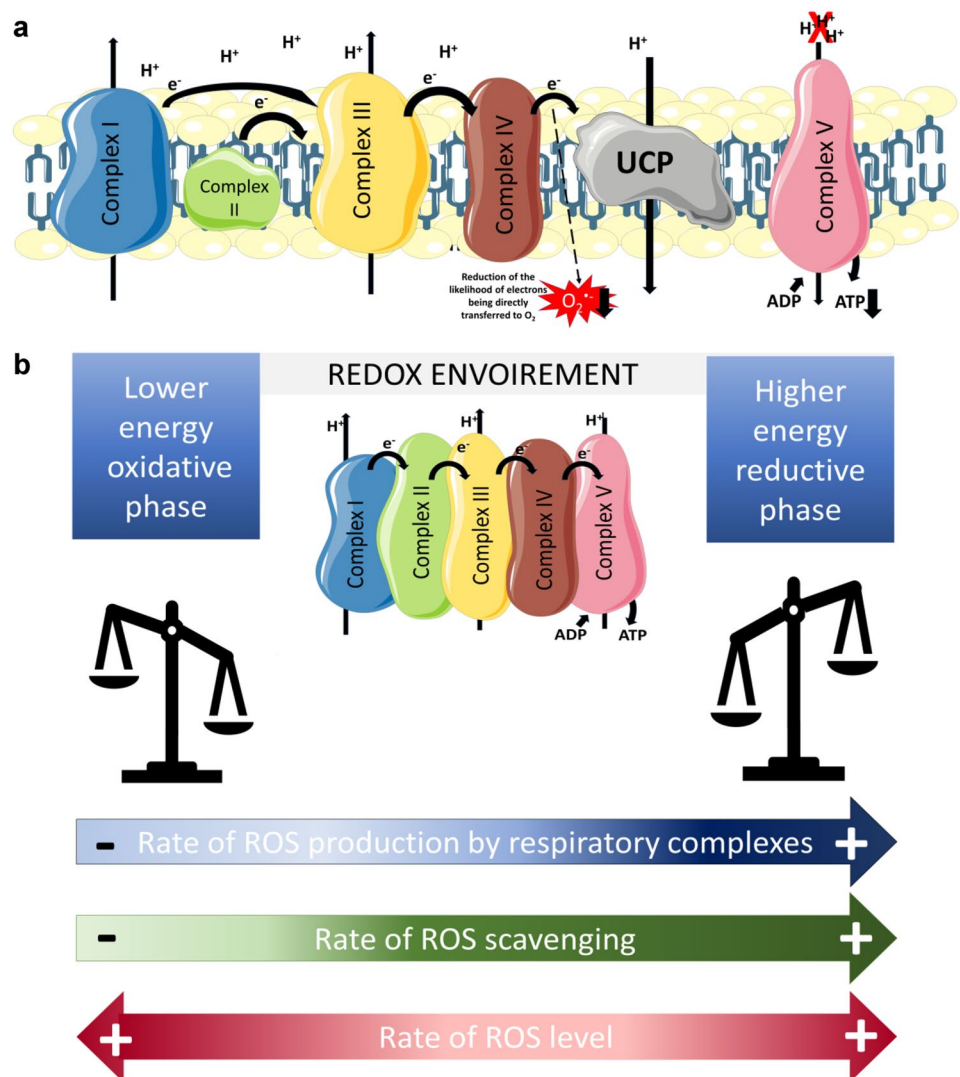
Proton leakage occurs mainly at complex I and complex III and could significantly compromise the magnitude of  $\Delta\Psi\text{m}$ . The equilibrium of  $\Delta\Psi\text{m}$  is essential to keep a normal mitochondrial function. An excessively high mitochondrial  $\Delta\Psi\text{m}$  is potentially harmful to mitochondria and consequently to the cell, since an excessive amount of ROS is produced and could directly cause various pathologies. On the other hand, at inappropriately low values of  $\Delta\Psi\text{m}$ , there is an inability to produce enough ATP, and the low mitochondrial ROS production could lead to the so-called 'reductive stress', which could be as detrimental to homeostasis as oxidative stress [147].

There are two main hypotheses to explain the mechanistic link between mitochondrial respiration and ROS levels: (1) ‘uncoupling to survive’ or ‘mild uncoupling’ and (2) redox-optimized ROS balance. The main difference between both is the proposed intermediary between respiration and ROS: mitochondrial membrane potential [ $\Delta\Psi_m$ ] and redox environment, respectively. In the former, it is postulated that mild uncoupling is a physiological feedback mechanism to prevent ROS by slightly dropping  $\Delta\Psi_m$  and increasing UCPs expression, thereby improving electrons transfers in the ETC and reducing the likelihood of electrons being directly transferred to  $O_2$  (Fig. 6a) [153, 155–157]. However, this premise has been only verified in isolated mitochondria, while, in vivo, cardiomyocytes from guinea pig ‘mild uncoupling’ elicited an increase rather than a decrease in ROS [152, 156, 158].

In fact, mild uncoupling was only confirmed in supra-physiologic redox potentials [158]. Furthermore, the reduction of ROS production by uncoupling occurs during state

4 of mitochondrial respiration, but cardiac mitochondria are never in state 4. Thus, pathological ROS production in cardiomyocytes is likely to be more closely linked to decreased or collapsed membrane potential and/or depletion of the NADPH pool [159], whereby ROS production overwhelms endogenous scavenging through mitochondrial membrane-dependent mechanisms.[137]. In a normal state, mitochondria maximize energy output while keeping hydrogen peroxide ( $H_2O_2$ ) overflow to a minimum by operating in an intermediate redox state [159]. According to redox-optimized ROS balance, the equilibrium of ROS is lost when the redox couples involved in electron transport (NADH/NAD<sup>+</sup>) or ROS scavenging (NADPH/NADP<sup>+</sup>, GSH/GSSG) are unbalanced toward the extremes of reduction or oxidation, respectively [158]. When the electrons flow slowly through the respiratory chain and redox environment is much reduced, the probability of generating the free radical superoxide increases irrespective of high antioxidant availability, but when the redox environment is rather

**Fig. 6** Two main hypotheses to explain the relationship between mitochondrial respiration and ROS production: **a** uncoupling to survive or mild uncoupling; **b** redox-optimized ROS balance





oxidized, the compromised scavenging capacity becomes rate-limiting, thus ROS overflow occurs (Fig. 6b).

As we move away from the ideal redox potential, a considerable amount of ROS outbreaks come up, as repeatedly shown in HFpEF [106, 160–163]. In several scenarios mitochondrial ROS can contribute to the deterioration of cardiac function, impairing ECC, causing arrhythmias, activating pro-hypertrophic signalling, and inducing apoptotic and/or necrotic cell death through activation of the permeability transition pore (PTP) [33, 159]

Chronic  $\beta$ -adrenergic stimulation induces mitochondrial ROS production, a process that can be amplified by the formation of aminochromes, catecholamine metabolites known to impair mitochondrial redox balance [137]. In experimental HFpEF animal models secondary to chronic pressure overload or angiotensin II administration, the increases of mitochondrial ROS can damage mitochondria and induce its dysfunction. Furthermore, cardiac dysfunction has been described as a consequence of ROS-induced mitochondrial dysfunction (decreased membrane potential, increased protein oxidation, and deletions in mitochondrial DNA (mtDNA)) and ROS-induced activation of the MAPK pathways [128, 164]. Effectively, increased ROS can lead to extensive oxidative damages in a variety of molecules, such as proteins, DNA, and lipids (including the ones presents in mitochondria) and modulate several signalling pathways involved in ventricular hypertrophy progression, contractile dysfunction,  $\text{Ca}^{2+}$  dysregulation, and myocardium stiffness [80, 106, 146]. As an example, ROS-increases PP-2a activity, decreasing PLB phosphorylation and reducing SERCA-2a activity, thus reducing  $\text{Ca}^{2+}$  reuptake to RS (Fig. 7) [165]. By modifying microRNA that leads to inhibition of SERCA2a transcription, diastole can also be impaired directly by ROS [166].

### Reactive oxygen species, nitric oxide and reactivenitrogen species

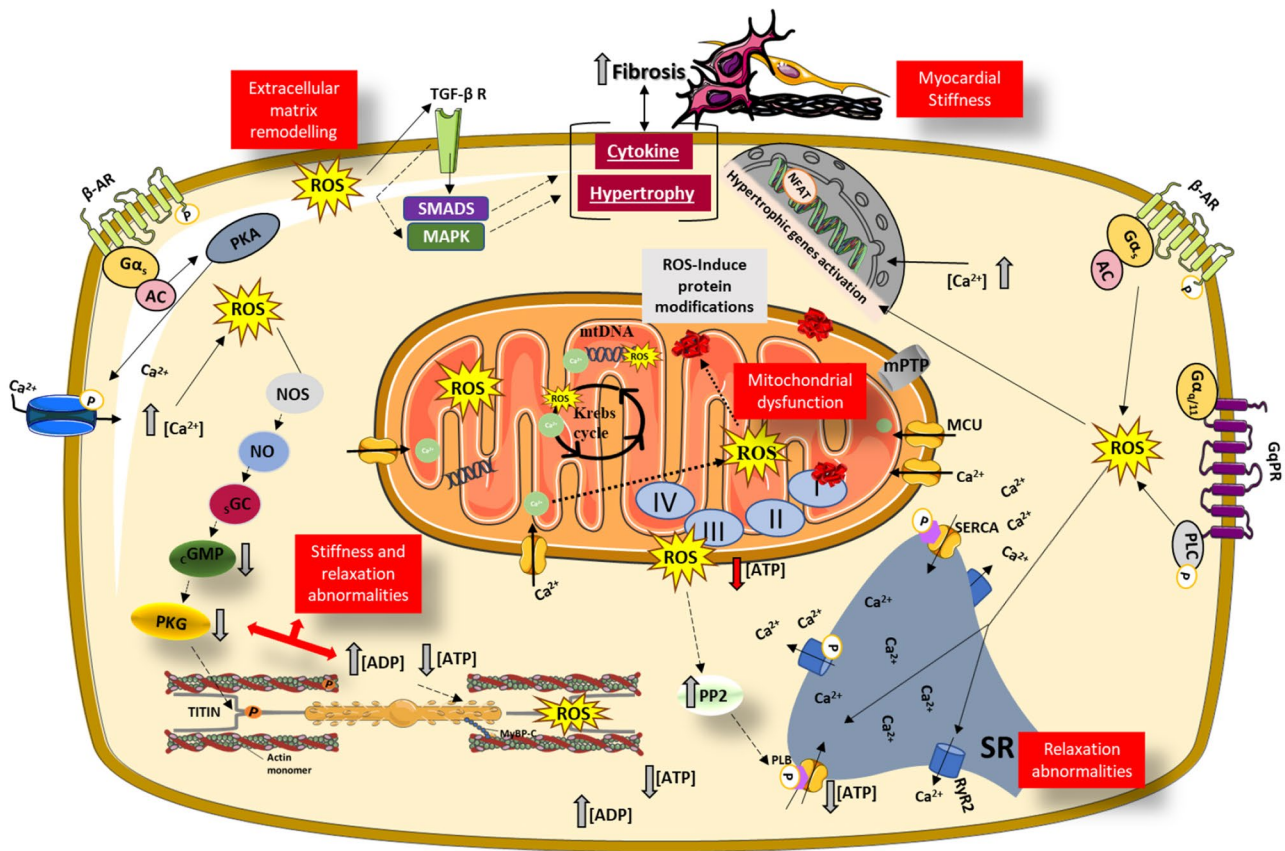
In the presence of ROS, nitric oxide (NO) and nitric oxide synthase (NOS) are oxidized, decreasing NO bioavailability [167] and consequently its beneficial effects including the control of mitochondrial pore opening [168]. The potent vasodilator NO is regulated by haemoglobin and myoglobin as well as by  $\text{O}_2$  gradients in the myocardium—when  $\text{O}_2$  concentrations are high, NO production decreases and when  $\text{O}_2$  concentrations decrease, NO increases in order to properly adjust blood flow to the tissue metabolic needs [168]. In the presence of low  $\text{O}_2$  concentrations, NO binds reversibly to  $\text{O}_2$  binding site on cytochrome c oxidase, adjusting the mitochondrial  $\text{O}_2$  consumption to its tissue content. In HF, the dysregulation of this mechanism is associated with impaired myocardial  $\text{O}_2$  consumption (MVO<sub>2</sub>) and the progression of compensated to decompensated HF [169]. High amounts

of NO or its derivatives, such as reactive nitrogen species (RNS), regulate oxidative phosphorylation by inhibiting mitochondrial respiration by two distinct mechanisms: (1) NO inhibition of cytochrome c oxidase, in an acute, potent and reversible manner through competition with  $\text{O}_2$  or (2) irreversible and generalized inhibition by RNS [170]. The latter occurs by RNS-induced post-translational modifications in these proteins, such as nitrosylation or oxidation of thiol groups and removal of iron from iron-sulfur centres [171]. As a consequence, the production of ATP decreases, while ROS and RNS increase, causing significant changes in myocardial intracellular signalling pathways and contributing to cell death [168]. In aortic constriction-induced hypertrophy, a concomitant increase in NO sensitivity in complex IV of the respiratory chain and an augment of inducible nitric oxide synthase (iNOS) has been described [172]. Additionally, post-translational modifications induced by ROS and RNS can cause alterations in Krebs cycle enzymes (e.g., aconitase) or inhibit enzymes such as CK [168].

In eukaryotes cells the central regulator of cellular metabolism is AMP-activated protein kinase (AMPK), which is activated when intracellular ATP levels lower [173]. AMPK plays critical roles in regulating growth and reprogramming metabolism and has recently been connected to cellular processes including mitochondrial biogenesis. Together with NO, AMPK can modulate mitochondrial biogenesis via cGMP or by enhancing the stimulation of transcription factors, such as PGC-1 $\alpha$ , NRF-1, or mtTFA, resulting in increased expression of respiratory chain complexes [168]. In HFpEF, the reduction NO bioavailability can imbalance the equilibrium between ROS and NO, impair the cGMP-PKG pathway, and contribute to the hypertrophic remodeling [160, 174]. As in HFpEF, PKG activity phosphorylate titin increasing its compliance, the NO depletion can contribute to increased cardiomyocyte passive tension (Fig. 7) [175, 176]. In contrast, the replacement of NO availability with BH<sub>4</sub> (a cofactor of NO synthesis) or activating NO-cGMP-PKG pathway improved DD and reduced the sensitivity of the myofilaments to  $\text{Ca}^{2+}$  [107, 177, 178]. In addition to uncoupled NOS, endothelial dysfunction further decreases NO bioavailability [179]. Overproduction of ROS/RNS from endothelial cells and decreased NO bioavailability in the myocardium have recently been shown to be important determinants of myocardial disease progression in both hypertensive and HFpEF patients [160, 179].

### Reactive oxygen species, inflammation and their impact on the extracellular matrix

Endothelial dysfunction and oxidative stress seems to be important mediators of inflammation, which are present in both HF subtypes [180]. In aortic valve disease, the rise of inflammatory mediators in endothelial cells increases



**Fig. 7** The impact of mitochondrial dysfunction and ROS production on diastolic function. The myocardial energetic dysfunction underlying the change of oxidative substrate leads to mitochondrial dysfunction. Mitochondrial dysfunction culminates in increased ROS, which, by decreasing NO and NOS bioavailability, reduce PKG and phosphorylation of titin. Together with titin hypophosphorylation, ROS act directly on titin, promote matrix remodelling, and release of proinflammatory cytokines. All these events culminate in increasing myocardial stiffness. Increased ROS-mediated protein phosphatase 2 (PP2) activity inhibits phospholamban phosphorylation and decreases SERCA-2a activity, thereby impairing relaxation. ROS also acts on ryanodine receptors promoting calcium leak. Mitochondrial proteins

released from the intermembrane space, such as Smac, cytochrome C, and apoptosis-inducing factor, lead to DNA fragmentation and trigger apoptotic events. Mitochondrial dysfunction causes an energetic deficit in the cell, affecting directly active relaxation. ADP, adenosine diphosphate; ATP, adenosine triphosphate; cGMP, cyclic guanosine monophosphate; GqPR, Gq-protein coupled receptor; MMPs, matrix metalloproteinases; NO, nitric oxide; NOS, nitric oxide synthase; PLB, phospholamban; PP2, protein phosphatase 2a; ROS, reactive oxygen species; PKG, protein kinase G; RyR, ryanodine receptors; sGC, soluble guanylate cyclase; SR, sarcoplasmic reticulum; TIMP, tissue inhibitors of matrix metalloproteinases; and TGF- $\beta$ , transforming growth factor-beta

oxidative stress and triggers the activation of the osteogenic and fibrogenic pathways, promoting valve calcification, and thus increasing LV afterload [181]. In the 1990s, inflammation and fibrosis were shown to promote LV hypertrophy. More recently, oxidative stress was proposed as an activator of transforming growth factor- $\beta$  (TGF- $\beta$ ) increasing collagen synthesis and inhibiting its degradation (Fig. 7) [182, 183]. Collagen is a crucial element of the extracellular matrix (ECM), essential to maintain myocardial structure, to assist mechanotransduction and, together with other proteins such as elastin, contribute to the elastic properties of the heart [109].

In HFpEF, ECM alterations result from changes in collagen metabolism, mainly in type I and type III collagen,

which are the most abundant in cardiac tissue (> 90%). These changes include alterations in its deposition, degree of cross-bridging, and relative amount, resulting in the development of myocardial interstitial fibrosis and subsequent ECM stiffening [184]. The activation of myofibroblasts that produces fibrosis is mostly due to the activation of renin-angiotensin-aldosterone system (RAAS) [185], a central neurohumoral axis in HFpEF [186]. Collagen metabolism results from the balance between metalloproteinases activity (MMPs) and their tissue inhibitors, TIMPs, and other proteins. In hypertensive patients with HFpEF, a downregulation of MMPs and an overexpression of TIMPs [187] have been described, resulting in a decrease in ECM degradation that contributes to increased fibrosis and thus,

ventricular stiffening (Fig. 7) [110]. In HFpEF patients, the presence of cells with an inflammatory profile leads to the release of factors, such as TGF- $\beta$ , responsible for inducing collagen production and promoting fibroblasts to myofibroblasts differentiation. By inducing an augment of cytokines and chemokines TGF- $\beta$  stimulates the recruitment and further activation of inflammatory cells, perpetuating a pro-inflammatory cycle and the progression of cardiac pathology. Compensatory hypertrophy has been shown augmented MCP-1, TGF- $\beta$ , interleukins (IL-6, IL-18 e IL-17R), TNF- $\alpha$ , anaphylatoxin C5a, or ST-2, which have been described to be involved in DD progression. Some markers of fibrosis and systemic inflammation such as galectin-3 and growth differentiating factor 15 (GDF15) have arisen as promising indicators of HFpEF progression [110]. Vascular cell adhesion molecule (VCAM, ICAM-1, and E-selectin), cells expressing inflammatory markers (CD3, CD11a, and CD45) with evidence of increased macrophage recruitment and increased activity of NOX2 within the recruited macrophages, were described to be upregulated in the myocardium from HFpEF patients [19].

## Therapeutic options

Despite the uncounted efforts to discover an efficient drug for HFpEF patients so far, there is no adequate therapeutic option. Several clinical trials have failed to demonstrate recovery of these patients. The undiscovered mechanism underlying the ventricular remodelling and reverse remodelling in HFpEF accounts significantly to this failure. Thus, the current treatment is largely directed toward improving HFpEF associated conditions (e.g., hypertension, DM; obesity) and symptoms (e.g., oedema). However, some promising new drugs are currently being tested.

## Traditional therapeutic approaches

The classical drugs to treat HF persist in the guidelines and are currently widely used in HFpEF, aiming at reducing symptoms, improving functional capacity, enhancing the quality of life and delaying the progression of the disease. Drugs that act on loop diuretics or inhibit neurohormonal activation and  $\beta$ -blockers have proven to interrupt the cycle of systolic dysfunction and improve survival of HF patients [188], while in HFpEF patients with pulmonary hypertension,  $\beta$ -adrenergic agonist seems to be beneficial by eliciting a robust vasodilator effect [189, 190].

In HFpEF clinical trials most of the drugs above mentioned showed incongruent results (supplement 1). Somewhat surprisingly, the drugs that proved to be efficient in HFrEF have not shown similar benefits in patients with

HFpEF. Furthermore, the disparity between observational and clinical studies probably reflects the differences in patients' characteristics enrolled in the studies. Together, the experimental and clinical studies suggest the need to abolish 'one size fits all approach' in searching a treatment for HFpEF and direct the pharmacological treatment according to the subgroup of HFpEF patients [191].

## Ionic-channel modulation

In HFpEF the excessive cardiomyocyte  $\text{Ca}^{2+}$  accumulation leads to elevated diastolic  $\text{Ca}^{2+}$  and increases resting tension, slower  $\text{Ca}^{2+}$  reuptake kinetics with impaired relaxation and impairs  $\text{Ca}^{2+}$  transients [192]. Cardiomyocyte  $\text{Ca}^{2+}$  accumulation is particularly pronounced when SERCA expression is reduced or its function impaired by post-translational induced by glycative or oxidative stress, affecting  $\text{Ca}^{2+}$  sequestration rate [191]. A class of drugs that promise to improve/normalize  $\text{Ca}^{2+}$  reuptake have been tested in HFpEF but the results were not promising (Supplementary Table 1).

When in excess mitochondrial  $\text{Ca}^{2+}$  accumulation can impair mitochondrial function, leading to reduced ATP production and increased release ROS [193]. As in HF, mitochondrial  $\text{Ca}^{2+}$  accumulation disturbs ion homeostasis and unbalances mitochondria redox state. Thus, improving mitochondria metabolism seems a valuable therapeutic option. An attractive approach would be targeting mitochondrial ion transporters. Indeed, the mechanisms of action of some peptides, such as preclinical CGP-37157, rely on mitochondrial  $\text{Ca}^{2+}$  regulation. By selectively inhibit mitochondrial NCLX, CGP-37157 maintain  $\text{Ca}^{2+}$  inside the mitochondria while ranolazine and empagliflozin reduce intracellular  $\text{Na}^+$  uptake, thus decreasing intracellular and mitochondrial  $\text{Ca}^{2+}$  [194–196]. Several ongoing clinical trials and further preclinical work should decipher whether the drugs that target mitochondria are beneficial and safe to use in HFpEF patients (Supplements 1).

## ROS and mitochondria

Other ions, such as iron, have been recently found to be reduced in HFpEF, and its deficiency could contribute to the disease development, probably through increased oxidative stress and reduced mitochondrial function, but evidence is scarce. It is postulated that the excess of iron content in mitochondria is connected to cytosolic iron depletion. Maintenance of mitochondrial iron homeostasis and the redistribution of iron using available iron chelators such as deferiprone and idebenone could result in the inhibition of free radical formation and partially

reverse LVRR in HFpEF as reported in cardiomyopathy of patients with Friedrich ataxia [197, 198].

Despite the undeniable imbalance of ROS in HFpEF and its deleterious impact on health, on clinical trials, ROS scavenging repeatedly fails to improve HF, which is consistent with the ROS hormesis concept [128]. This concept suggests that in HF, antioxidants per se are not a plausible mechanism of action for long-term improvement. Several studies have noted that administration of high doses of ROS scavengers can even abolish the beneficial effects of exercise [137]. However, the lack of tissue permeability, poor intra-cellular targeting, or ineffective therapeutic doses may underlie the lack of positive results to date [199, 200].

Novel scavenging compounds that directly targeted mitochondria, such as Elamipretide, showed promising clinical or pre-clinical results by improving myocardial high energy phosphates; reducing mitochondrial oxidative stress and damage; ameliorating skeletal muscle function, exercise capacity, cardiac hypertrophy, and systolic and diastolic function; and reducing myocardial fibrosis and cardiovascular mortality [197]. This new class of aromatic-cationic and cell-permeable tetrapeptides that stabilize cardiolipin and improve coupling of the ETC is currently under investigation (supplement 1) [21, 137, 201–203].

In a higher oxidative environment, NO availability decreases, reducing its beneficial effects. Some approaches to increase NO through the sGC system and its downstream signalling pathways using phosphodiesterase-5 inhibitors and Vericiguat have been tested. Targeting the nitrate-nitrite-NO pathway with inhaled nitrites failed first to improve quality of life or aerobic capacity, while orally administered inorganic nitrate shown encouraging results (Supplement 1) [204].

### Modulation of oxidative substrate

Another promising strategy for HFpEF treatment is based on the oxidative substrate modulation. In HFpEF the use of inhibitors of FA  $\beta$ -oxidation or the stimulators of glucose oxidation has been demonstrated to improve haemodynamic parameters (supplement 1) [205]. Furthermore, trimetazidine has been included in the 2016 European guidelines for the HF treatment because it improves functional capacity, delays or reverses LV remodelling and dysfunction, and reduces BNP levels [206]. Improving the coupling of glycolysis and glucose oxidation may be a promising target for the treatment of diseases characterized by abnormal cell growth, such as hypertrophic remodelling [60, 75][207].

Inversely, PDK4 induces a shift on oxidative substrate favouring FA catabolism, and in LV pressure overload,

its activation promotes the uncoupling of glucose uptake/oxidation and induces G6P accumulation and ER stress. Together these mechanisms contributed to LV hypertrophy and DD development and [59, 76] exacerbated the cardiomyopathy secondary to calcineurin stress-activated pathway [208]. However, PDK inhibition partially restored right ventricle function and attenuated hypertrophy by restoring right ventricle repolarization [207].

Another class of natural compounds that acts as bio-energetic enhancers, resveratrol, a polyphenol found in red wine showed to be able to reduce cardiac dysfunction and improve mitochondrial biogenesis in hypertension-mediated HF by increasing AMPK. In humans that had diastolic HF, grape seed extract decreased systolic and diastolic blood pressures [21]. Ubiquinol and D-ribose proved to have physiological benefits by promoting cardiac energy metabolism and increasing mitochondrial ATP regeneration. Ubiquinol improves ETC electron flow, increasing proton gradient and thus ADP phosphorylation, while D-ribose provides a source for the synthesis of adenosine monophosphate (AMP) to ensure there is enough ADP to form ATP. Until now, several approaches to modulate or improve cardiac metabolism in HF have been tested, partial adenosine A1-agonists, carnitine palmitoyltransferase-1 inhibitors, FA-oxidation inhibitor, mitochondrial enhancer, and intravenous iron are being evaluated in patients with HFpEF or diastolic dysfunction (supplement 1) [209–211].

### Dietary modulation to treat HFpEF

Nutritional or behaviour interventions to restrict calorie intake or losing weight improve exercise tolerance and aerobic capacity in obese HFpEF patients [212]. However, the role of dietary components in HFpEF (or other diseases) remains largely unknown.

Sugar consumption is linked to obesity, diabetes, and impaired cardiorespiratory fitness in patients with HFpEF. Experimental models and preclinical studies confirmed the toxic effects of a high-sugar or Western-like diet that induce cardiac dysfunction, affecting mainly diastole [213]. Modulation/restriction of carbohydrate intake normalizes postprandial hyperglycemic index and insulinemic peaks and has been shown to improve all manifestations of the MetS as well as to reduce cardiovascular risk [214]. Similarly, in experimental or pre-clinical studies, increasing dietary unsaturated FA (UFA) and reducing sugars consumption had preserved myocardial function mainly diastole, independently of weight gain or calorie intake [215]. Conversely, UFAs exerts its beneficial effects by potentially abolished NLRP3 inflammasome activation and inhibiting the subsequent synthesis of pro-inflammatory cytokines [216].



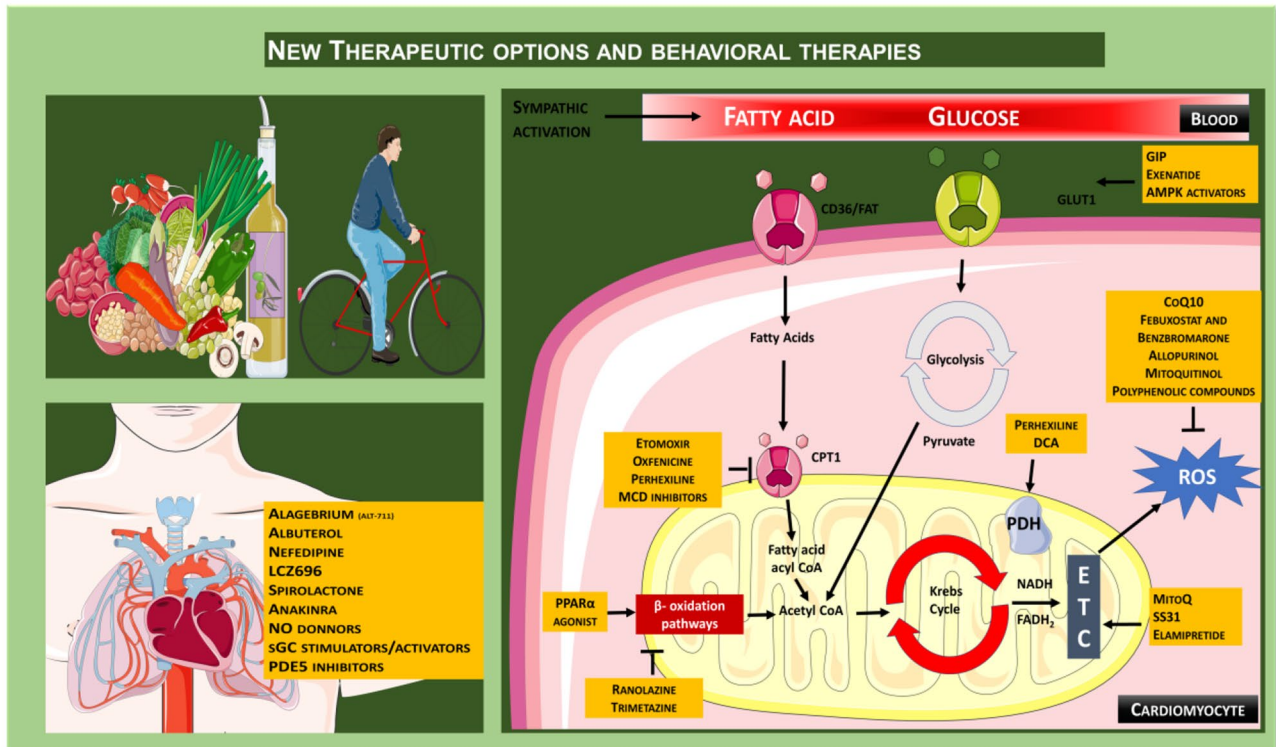
Controversially, in obese HFpEF patients the consumption and/or supplementation of unsaturated FA (UFA, monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs)) was associated with better aerobic capacity and positively correlated with improved diastolic function and with larger percentage of fat-free mass [215]. Furthermore, this kind of diet has been associated with lower major cardiovascular event and overall mortality rates in subjects with high cardiovascular risk, independent of the overall dietary caloric intake or body-weight [217]. These data highlight the ability of diet to modulate cardiac function in experimental animal settings, showing that the quality of nutrients rather than the amount of calories affects cardiac function, with a high-UFA, low-sugar content opposing the detrimental effects of saturated fat and sugars.

Thus, switching back to a ‘healthy’ diet and increasing physical activity offer a promising behaviour intervention that is currently under investigation (supplement 1) [213, 218]. As this kind of diet is difficult to accomplish and requires strict adherence to a high-fat and low-carbohydrate food, synthetic ketone ester drink ( $\Delta G^{\circ}$ ) have been developed. This drink has proven to be more effective in achieving ketogenesis than any other diet by reaching five-fold higher circulating D- $\beta$ OHB

levels, improving physical performance and even cognitive function [18, 219]. However, whether exogenous ketones have the same effect of nutritional endogenous ketosis is still a matter of debate.

## Conclusion

Throughout this paper, we provided an overview of the pathological mechanism and promising therapeutic options for HFpEF (Fig. 8). HFpEF remains an intriguing condition to clinicians and researchers as it aggregates more than a single pathology, representing a multifactorial and complex disease. Therefore, several mechanisms are expected to be implicated in this pathology but, so far, it has been impossible to establish an integrated view of HFpEF. This syndrome has long been recognized as a metabolic disease; however, the interest on metabolic disturbances associated with HFpEF has only recently gained interest. Nevertheless, due to the intricate nature of metabolic mechanisms, the current knowledge represents just the tip of the iceberg and has even encourage the recent proposal of a new HFpEF categorization in three distinct subclasses according to its specific phenotype: (1) young patients with moderate DD and normal BNP levels, (2) obese and diabetic patients with sleep apnoea that shown severe impairment of LV



**Fig. 8** Behavioural approaches and pharmacological therapies that has been proposed to treat HFpEF or improve symptoms or quality of life of those patients

relaxation, and (3) older patients with chronic kidney disease, myocardial dysfunction, and pulmonary hypertension [220]. The deconstruction of this complex syndrome will surely be of great value in the near future, improving HFpEF diagnosis and therapeutics and uncovering the submerged part of the iceberg.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Fukuta H, Little WC (2008) The cardiac cycle and the physiologic basis of left ventricular contraction, ejection, relaxation, and filling. *Heart failure clinics* 4(1):1–11. <https://doi.org/10.1016/j.hfc.2007.10.004>
- Gomes MC, Ferreira A, Bettencourt P (2004) [Physiopathology of heart failure]. *Revista portuguesa de cardiologia : Orgao oficial da Sociedade Portuguesa de Cardiologia = Portuguese journal of cardiology : An official journal of the Portuguese Society of Cardiology* 23 Suppl 2:ii7–23
- Stanley WC, Recchia FA, Lopaschuk GD (2005) Myocardial substrate metabolism in the normal and failing heart. *Physiol Rev* 85(3):1093–1129. <https://doi.org/10.1152/physrev.00006.2004>
- Mosterd A, Hoes AW (2007) Clinical epidemiology of heart failure Heart (British Cardiac Society) 93(9):1137–1146. <https://doi.org/10.1136/hrt.2003.025270>
- 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) 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. *European journal of heart failure* 18 (8):891–975. <https://doi.org/10.1002/ejhf.592>
- Lam CS, Donal E, Kraigher-Krainer E, Vasan RS (2011) Epidemiology and clinical course of heart failure with preserved ejection fraction. *Eur J Heart Fail* 13(1):18–28. <https://doi.org/10.1093/eurjhf/hfq121>
- Borlaug BA, Paulus WJ (2011) Heart failure with preserved ejection fraction: Pathophysiology, diagnosis, and treatment. *Eur Heart J* 32(6):670–679. <https://doi.org/10.1093/eurheartj/ehq426>
- Huis In 't Veld AE, de Man FS, van Rossum AC, Handoko ML (2016) How to diagnose heart failure with preserved ejection fraction: The value of invasive stress testing. *Netherlands heart journal : Monthly journal of the Netherlands Society of Cardiology and the Netherlands Heart Foundation* 24(4):244–251. <https://doi.org/10.1007/s12471-016-0811-0>
- Scheubel RJ, Tostlebe M, Simm A, Rohrbach S, Prondzinsky R, Gellerich FN, Silber RE, Holtz J (2002) Dysfunction of mitochondrial respiratory chain complex I in human failing myocardium is not due to disturbed mitochondrial gene expression. *J Am Coll Cardiol* 40(12):2174–2181
- Sinning C, Kempf T, Schwarzl M, Lanfermann S, Ojeda F, Schnabel RB, Zengin E, Wild PS, Lackner KJ, Munzel T, Blankenberg S, Wollert KC, Zeller T, Westermann D (2017) Biomarkers for characterization of heart failure—distinction of heart failure with preserved and reduced ejection fraction. *Int J Cardiol* 227:272–277. <https://doi.org/10.1016/j.ijcard.2016.11.110>
- Dai DF, Hsieh EJ, Chen T, Menendez LG, Basisty NB, Tsai L, Beyer RP, Crispin DA, Shulman NJ, Szeto HH, Tian R, MacCoss MJ, Rabinovitch PS (2013) Global proteomics and pathway analysis of pressure-overload-induced heart failure and its attenuation by mitochondrial-targeted peptides. *Circulation Heart failure* 6(5):1067–1076. <https://doi.org/10.1161/CIRCHEARTFAILURE.113.000406>
- Shi J, Dai W, Hale SL, Brown DA, Wang M, Han X, Kloner RA (2015) Bendavia restores mitochondrial energy metabolism gene expression and suppresses cardiac fibrosis in the border zone of the infarcted heart. *Life Sci* 141:170–178. <https://doi.org/10.1016/j.lfs.2015.09.022>
- Rodrigues PG, Leite-Moreira AF, Falcao-Pires I (2016) Myocardial reverse remodeling: how far can we rewind? *Am J Physiol Heart Circ Physiol* 310(11):H1402–1422. <https://doi.org/10.1152/ajpheart.00696.2015>
- Gavina C, Falcao-Pires I, Rodrigues J, Marinho B, Goncalves N, Lopes R, Amorim MJ, Almeida J, Pinho P, Goncalves A, Rocha-Goncalves F, Leite-Moreira A (2014) Load independent impairment of reverse remodeling after valve replacement in hypertensive aortic stenosis patients. *Int J Cardiol* 170(3):324–330. <https://doi.org/10.1016/j.ijcard.2013.11.006>
- Campbell KS, Sorrell VL (2015) Cell- and molecular-level mechanisms contributing to diastolic dysfunction in HFpEF. *J Appl Physiol* 119(10):1228–1232. <https://doi.org/10.1152/jappphysiol.01168.2014>
- Schirone L, Forte M, Palmerio S, Yee D, Nocella C, Angelini F, Pagano F, Schiavon S, Bordin A, Carrizzo A, Vecchione C, Valenti V, Chimenti I, De Falco E, Sciarretta S, Frati G (2017) A review of the molecular mechanisms underlying the development and progression of cardiac remodeling. *Oxidative medicine and cellular longevity* 2017:3920195. <https://doi.org/10.1155/2017/3920195>
- Rouhana S, Farah C, Roy J, Finan A (1865) Rodrigues de Araujo G, Bideaux P, Scheuermann V, Saliba Y, Reboul C, Cazorla O, Aimond F, Richard S, Thireau J, Fares N (2019) Early calcium handling imbalance in pressure overload-induced heart failure with nearly normal left ventricular ejection fraction. *Biochim Biophys Acta* 1:230–242. <https://doi.org/10.1016/j.bbadis.2018.08.005>
- Maack C, Lehrke M, Backs J, Heinzl FR, Hulot JS, Marx N, Paulus WJ, Rossignol P, Taegtmeier H, Bauersachs J, Bayes-Genis A, Brutsaert D, Bugger H, Clarke K, Cosentino F, De Keulenaer G, Dei Cas A, Gonzalez A, Huelsmann M, Iaccarino G, Lunde IG, Lyon AR, Pollesello P, Rena G, Riksen NP, Rosano G, Staels B, van Laake LW, Wanner C, Farmakis D, Filippatos G, Ruschitzka F, Seferovic P, de Boer RA, Heymans S (2018) Heart failure and diabetes: metabolic alterations and therapeutic interventions: A state-of-the-art review from the Translational Research Committee of the Heart Failure Association-European

- Society of Cardiology. *Eur Heart J* 39(48):4243–4254. <https://doi.org/10.1093/eurheartj/ehy596>
19. Plitt GD, Spring JT, Moulton MJ, Agrawal DK (2018) Mechanisms, diagnosis, and treatment of heart failure with preserved ejection fraction and diastolic dysfunction. *Expert review of cardiovascular therapy* 16(8):579–589. <https://doi.org/10.1080/14779072.2018.1497485>
  20. Kasner M, Aleksandrov AS, Westermann D, Lassner D, Gross M, von Haehling S, Anker SD, Schultheiss HP, Tschope C (2013) Functional iron deficiency and diastolic function in heart failure with preserved ejection fraction. *Int J Cardiol* 168(5):4652–4657. <https://doi.org/10.1016/j.ijcard.2013.07.185>
  21. Kumar AA, Kelly DP, Chirinos JA (2019) Mitochondrial dysfunction in heart failure with preserved ejection fraction. *Circulation* 139(11):1435–1450. <https://doi.org/10.1161/CIRCULATIONAHA.118.036259>
  22. Bates A, Shen Q, Hiebert JB, Thimmesch A, Pierce JD (2014) Myocardial energetics and ubiquinol in diastolic heart failure. *Nursing & health sciences* 16(4):428–433. <https://doi.org/10.1111/nhs.12168>
  23. Luptak I, Qin F, Sverdlow AL, Pimentel DR, Panagia M, Croteau D, Siwik DA, Bachschmid MM, He H, Balschi JA, Colucci WS (2019) Energetic dysfunction is mediated by mitochondrial reactive oxygen species and precedes structural remodeling in metabolic heart disease. *Antioxid Redox Signal* 31(7):539–549. <https://doi.org/10.1089/ars.2018.7707>
  24. Koncos G, Varga ZV, Baranyai T, Boengler K, Rohrbach S, Li L, Schluter KD, Schreckenberger R, Radovits T, Olah A, Matyas C, Lux A, Al-Khrasani M, Komlodi T, Bukosza N, Mathe D, Deres L, Bartekova M, Rajtik T, Adameova A, Szigeti K, Hamar P, Helyes Z, Tretter L, Pacher P, Merkely B, Giricz Z, Schulz R, Ferdinandy P (2016) Diastolic dysfunction in prediabetic male rats: Role of mitochondrial oxidative stress. *Am J Physiol Heart Circ Physiol* 311(4):H927–H943. <https://doi.org/10.1152/ajpheart.00049.2016>
  25. 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 (2009) 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* 54(5):402–409. <https://doi.org/10.1016/j.jacc.2009.05.012>
  26. Landstrom AP, Dobrev D, Wehrens XHT (2017) Calcium signaling and cardiac arrhythmias. *Circ Res* 120(12):1969–1993. <https://doi.org/10.1161/CIRCRESAHA.117.310083>
  27. Mayourian J, Ceholski DK, Gonzalez DM, Cashman TJ, Sahoo S, Hajjar RJ, Costa KD (2018) Physiologic, pathologic, and therapeutic paracrine modulation of cardiac excitation-contraction coupling. *Circ Res* 122(1):167–183. <https://doi.org/10.1161/CIRCRESAHA.117.311589>
  28. Cao JL, Adaniya SM, Cypress MW, Suzuki Y, Kusakari Y, Jhun BS, J OU, (2019) Role of mitochondrial Ca(2+) homeostasis in cardiac muscles. *Arch Biochem Biophys* 663:276–287. <https://doi.org/10.1016/j.abb.2019.01.027>
  29. Maack C, Cortassa S, Aon MA, Ganesan AN, Liu T, O'Rourke B (2006) Elevated cytosolic Na+ decreases mitochondrial Ca2+ uptake during excitation-contraction coupling and impairs energetic adaptation in cardiac myocytes. *Circ Res* 99(2):172–182. <https://doi.org/10.1161/01.RES.0000232546.92777.05>
  30. Maack C, O'Rourke B (2007) Excitation-contraction coupling and mitochondrial energetics. *Basic Res Cardiol* 102(5):369–392. <https://doi.org/10.1007/s00395-007-0666-z>
  31. De La Fuente S, Lambert JP, Nichtova Z, Fernandez Sanz C, Elrod JW, Sheu SS, Csordas G (2018) Spatial Separation of Mitochondrial Calcium Uptake and Extrusion for Energy-Efficient Mitochondrial Calcium Signaling in the Heart. *Cell reports* 24 (12):3099–3107 e3094. <https://doi.org/10.1016/j.celrep.2018.08.040>
  32. Pinton P, Giorgi C, Siviero R, Zecchini E, Rizzuto R (2008) Calcium and apoptosis: ER-mitochondria Ca2+ transfer in the control of apoptosis. *Oncogene* 27(50):6407–6418. <https://doi.org/10.1038/onc.2008.308>
  33. Olver TD, Edwards JC, Jurrissen TJ, Veteto AB, Jones JL, Gao C, Rau C, Warren CM, Klutho PJ, Alex L, Ferreira-Nichols SC, Ivey JR, Thorne PK, McDonald KS, Krenz M, Baines CP, Solaro RJ, Wang Y, Ford DA, Domeier TL, Padilla J, Rector RS, Emter CA (2019) Western diet-fed, aortic-banded ossabaw swine: A preclinical model of cardio-metabolic heart failure. *JACC Basic to translational science* 4(3):404–421. <https://doi.org/10.1016/j.jacbs.2019.02.004>
  34. Rizzuto R, Duchon MR, Pozzan T (2004) Flirting in little space: the ER/mitochondria Ca2+ liaison. *Science's STKE : Signal transduction knowledge environment* 2004 (215):re1. <https://doi.org/10.1126/stke.2152004re1>
  35. Giacomello M, Drago I, Bortolozzi M, Scorzeto M, Gianelle A, Pizzo P, Pozzan T (2010) Ca2+ hot spots on the mitochondrial surface are generated by Ca2+ mobilization from stores, but not by activation of store-operated Ca2+ channels. *Mol Cell* 38(2):280–290. <https://doi.org/10.1016/j.molcel.2010.04.003>
  36. Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA, Pozzan T (1998) Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca2+ responses. *Science* 280(5370):1763–1766. <https://doi.org/10.1126/science.280.5370.1763>
  37. Fearnley CJ, Roderick HL, Bootman MD (2011) Calcium signaling in cardiac myocytes. *Cold Spring Harbor perspectives in biology* 3(11):a004242. <https://doi.org/10.1101/cshperspect.a004242>
  38. Bers DM (2002) Cardiac excitation-contraction coupling. *Nature* 415(6868):198–205. <https://doi.org/10.1038/415198afpii>
  39. Bertero E, Maack C (2018) Calcium signaling and reactive oxygen species in mitochondria. *Circ Res* 122(10):1460–1478. <https://doi.org/10.1161/CIRCRESAHA.118.310082>
  40. Loffredo FS, Nikolova AP, Pancoast JR, Lee RT (2014) Heart failure with preserved ejection fraction: Molecular pathways of the aging myocardium. *Circ Res* 115(1):97–107. <https://doi.org/10.1161/CIRCRESAHA.115.302929>
  41. Bers DM, Despa S (2009) Na+ transport in cardiac myocytes. Implications for excitation-contraction coupling *IUBMB life* 61(3):215–221. <https://doi.org/10.1002/iub.163>
  42. Peana D, Domeier TL (2017) Cardiomyocyte Ca(2+) homeostasis as a therapeutic target in heart failure with reduced and preserved ejection fraction. *Curr Opin Pharmacol* 33:17–26. <https://doi.org/10.1016/j.coph.2017.03.005>
  43. Primessnig U, Schonleitner P, Holl A, Pfeiffer S, Bracic T, Rau T, Kapl M, Stojakovic T, Glasnov T, Leineweber K, Wakula P, Antoons G, Pieske B, Heinzel FR (2016) Novel pathomechanisms of cardiomyocyte dysfunction in a model of heart failure with preserved ejection fraction. *Eur J Heart Fail* 18(8):987–997. <https://doi.org/10.1002/ejhf.524>
  44. Richard S, Leclercq F, Lemaire S, Piot C, Nargeot J (1998) Ca2+ currents in compensated hypertrophy and heart failure. *Cardiovasc Res* 37(2):300–311. [https://doi.org/10.1016/s0008-6363\(97\)00273-3](https://doi.org/10.1016/s0008-6363(97)00273-3)
  45. Flarsheim CE, Grupp IL, Matlib MA (1996) Mitochondrial dysfunction accompanies diastolic dysfunction in diabetic rat heart. *The American journal of physiology* 271(1 Pt 2):H192–202. <https://doi.org/10.1152/ajpheart.1996.271.1.H192>
  46. Tian R, Ingwall JS (1996) Energetic basis for reduced contractile reserve in isolated rat hearts. *Am J Physiol* 270(4 Pt 2):H1207–1216. <https://doi.org/10.1152/ajpheart.1996.270.4.H1207>
  47. Eleid MF, Nishimura RA, Sorajja P, Borlaug BA (2013) Systemic hypertension in low-gradient severe aortic stenosis with



- preserved ejection fraction. *Circulation* 128(12):1349–1353. <https://doi.org/10.1161/circulationaha.113.003071>
48. De Jong KA, Lopaschuk GD (2017) Complex energy metabolic changes in heart failure with preserved ejection fraction and heart failure with reduced ejection fraction. *The Canadian journal of cardiology* 33(7):860–871. <https://doi.org/10.1016/j.cjca.2017.03.009>
  49. Huss JM, Kelly DP (2005) Mitochondrial energy metabolism in heart failure: A question of balance. *J Clin Invest* 115(3):547–555. <https://doi.org/10.1172/jci24405>
  50. Doenst T, Nguyen TD, Abel ED (2013) Cardiac metabolism in heart failure: Implications beyond ATP production. *Circ Res* 113(6):709–724. <https://doi.org/10.1161/CIRCRESAHA.113.300376>
  51. Lionetti V, Stanley WC, Recchia FA (2011) Modulating fatty acid oxidation in heart failure. *Cardiovasc Res* 90(2):202–209. <https://doi.org/10.1093/cvr/cvr038>
  52. Taegtmeyer H, Sen S, Vela D (2010) Return to the fetal gene program: a suggested metabolic link to gene expression in the heart. *Ann N Y Acad Sci* 1188:191–198. <https://doi.org/10.1111/j.1749-6632.2009.05100.x>
  53. Sharma N, Okere IC, Brunengraber DZ, McElfresh TA, King KL, Sterk JP, Huang H, Chandler MP, Stanley WC (2005) Regulation of pyruvate dehydrogenase activity and citric acid cycle intermediates during high cardiac power generation. *The Journal of physiology* 562(Pt 2):593–603. <https://doi.org/10.1113/jphysiol.2004.075713>
  54. Kim JW, Tchernyshyov I, Semenza GL, Dang CV (2006) HIF-1-mediated expression of pyruvate dehydrogenase kinase: A metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* 3(3):177–185. <https://doi.org/10.1016/j.cmet.2006.02.002>
  55. Jensen MD (2003) Fate of fatty acids at rest and during exercise: Regulatory mechanisms. *Acta Physiol Scand* 178(4):385–390. <https://doi.org/10.1046/j.1365-201X.2003.01167.x>
  56. Opie LH (2004) The metabolic vicious cycle in heart failure. *The Lancet* 364:1733–1734
  57. Glatz JF, Angin Y, Steinbusch LK, Schwenk RW, Luiken JJ (2013) CD36 as a target to prevent cardiac lipotoxicity and insulin resistance. *Prostaglandins Leukot Essent Fatty Acids* 88(1):71–77. <https://doi.org/10.1016/j.plefa.2012.04.009>
  58. Jaswal JS, Keung W, Wang W, Ussher JR (2011) Lopaschuk GD (2011) Targeting fatty acid and carbohydrate oxidation—a novel therapeutic intervention in the ischemic and failing heart. *Biochem Biophys Acta* 7:1333–1350. <https://doi.org/10.1016/j.bbamcr.2011.01.015>
  59. Sen S, Kundu BK, Wu HC, Hashmi SS, Guthrie P, Locke LW, Roy RJ, Matherne GP, Berr SS, Terwelp M, Scott B, Carranza S, Frazier OH, Glover DK, Dillmann WH, Gambello MJ, Entman ML, Taegtmeyer H (2013) Glucose regulation of load-induced mTOR signaling and ER stress in mammalian heart. *Journal of the American Heart Association* 2(3):e004796. <https://doi.org/10.1161/JAHA.113.004796>
  60. Fillmore N, Levasseur JL, Fukushima A, Wagg CS, Wang W, Dyck JRB, Lopaschuk GD (2018) Uncoupling of glycolysis from glucose oxidation accompanies the development of heart failure with preserved ejection fraction. *Mol Med* 24(1):3. <https://doi.org/10.1186/s10020-018-0005-x>
  61. Karwi QG, Uddin GM, Ho KL, Lopaschuk GD (2018) Loss of metabolic flexibility in the failing heart. *Front Cardiovasc Med* 5:68. <https://doi.org/10.3389/fcvm.2018.00068>
  62. Fillmore N, Mori J, Lopaschuk GD (2014) Mitochondrial fatty acid oxidation alterations in heart failure, ischaemic heart disease and diabetic cardiomyopathy. *Br J Pharmacol* 171(8):2080–2090. <https://doi.org/10.1111/bph.12475>
  63. Liao R, Jain M, Cui L, D'Agostino J, Aiello F, Luptak I, Ngoy S, Mortensen RM, Tian R (2002) Cardiac-specific overexpression of GLUT1 prevents the development of heart failure attributable to pressure overload in mice. *Circulation* 106(16):2125–2131. <https://doi.org/10.1161/01.cir.0000034049.61181.f3>
  64. Grynberg A, Demaison L (1996) Fatty acid oxidation in the heart. *J Cardiovasc Pharmacol* 28(Suppl 1):S11–17. <https://doi.org/10.1097/00005344-199600003-00003>
  65. Kato T, Niizuma S, Inuzuka Y, Kawashima T, Okuda J, Tamaki Y, Iwanaga Y, Narazaki M, Matsuda T, Soga T, Kita T, Kimura T, Shioi T (2010) Analysis of metabolic remodeling in compensated left ventricular hypertrophy and heart failure. *Circulation Heart failure* 3(3):420–430. <https://doi.org/10.1161/CIRCHEARTFAILURE.109.888479>
  66. Gupte SA, Levine RJ, Gupte RS, Young ME, Lionetti V, Labinskyy V, Floyd BC, Ojaimi C, Bellomo M, Wolin MS, Recchia FA (2006) Glucose-6-phosphate dehydrogenase-derived NADPH fuels superoxide production in the failing heart. *J Mol Cell Cardiol* 41(2):340–349. <https://doi.org/10.1016/j.yjmcc.2006.05.003>
  67. Stanton RC (2012) Glucose-6-phosphate dehydrogenase, NADPH, and cell survival. *IUBMB Life* 64(5):362–369. <https://doi.org/10.1002/iub.1017>
  68. Akki A, Smith K, Seymour AM (2008) Compensated cardiac hypertrophy is characterised by a decline in palmitate oxidation. *Mol Cell Biochem* 311(1–2):215–224. <https://doi.org/10.1007/s11010-008-9711-y>
  69. Allard MF, Schonekess BO, Henning SL, English DR, Lopaschuk GD (1994) Contribution of oxidative metabolism and glycolysis to ATP production in hypertrophied hearts. *The American journal of physiology* 267(2 Pt 2):H742–750
  70. Degens H, de Brouwer KF, Gilde AJ, Lindhout M, Willemsen PH, Janssen BJ, van der Vusse GJ, van Bilsen M (2006) Cardiac fatty acid metabolism is preserved in the compensated hypertrophic rat heart. *Basic Res Cardiol* 101(1):17–26. <https://doi.org/10.1007/s00395-005-0549-0>
  71. Taylor M, Wallhaus TR, Degrado TR, Russell DC, Stanko P, Nickles RJ, Stone CK (2001) An evaluation of myocardial fatty acid and glucose uptake using PET with [18F] fluoro-6-thia-heptadecanoic acid and [18F] FDG in patients with congestive heart failure. *Journal of nuclear medicine : Official publication, Society of Nuclear Medicine* 42(1):55–62
  72. Young ME, Laws FA, Goodwin GW, Taegtmeyer H (2001) Reactivation of peroxisome proliferator-activated receptor alpha is associated with contractile dysfunction in hypertrophied rat heart. *The Journal of biological chemistry* 276(48):44390–44395. <https://doi.org/10.1074/jbc.M103826200>
  73. Hamirani YS, Kundu BK, Zhong M, McBride A, Li Y, Davogustto GE, Taegtmeyer H, Bourque JM (2016) Noninvasive detection of early metabolic left ventricular remodeling in systemic hypertension. *Cardiology* 133(3):157–162. <https://doi.org/10.1159/000441276>
  74. Noma T, Nishiyama A, Mizushige K, Murakami K, Tsuji T, Kohno M, Rahman M, Fukui T, Abe Y, Kimura S (2001) Possible role of uncoupling protein in regulation of myocardial energy metabolism in aortic regurgitation model rats. *FASEB journal : Official publication of the Federation of American Societies for Experimental Biology* 15(7):1206–1208
  75. Sankaralingam S, Lopaschuk GD (2015) Cardiac energy metabolic alterations in pressure overload-induced left and right heart failure (2013 Grover Conference Series). *Pulmonary circulation* 5(1):15–28. <https://doi.org/10.1086/679608>
  76. Mori J, Basu R, McLean BA, Das SK, Zhang L, Patel VB, Wagg CS, Kassiri Z, Lopaschuk GD, Oudit GY (2012) Agonist-induced hypertrophy and diastolic dysfunction are associated with selective reduction in glucose oxidation: a metabolic contribution to heart failure



- with normal ejection fraction. *Circulation Heart failure* 5(4):493–503. <https://doi.org/10.1161/CIRCHEARTFAILURE.112.966705>
77. Harmancey R, Lam TN, Lubrano GM, Guthrie PH, Vela D, Taegtmeier H (2012) Insulin resistance improves metabolic and contractile efficiency in stressed rat heart. *FASEB journal : Official publication of the Federation of American Societies for Experimental Biology* 26(8):3118–3126. <https://doi.org/10.1096/fj.12-208991>
  78. Lopaschuk GD, Ussher JR, Folmes CD, Jaswal JS, Stanley WC (2010) Myocardial fatty acid metabolism in health and disease. *Physiol Rev* 90(1):207–258. <https://doi.org/10.1152/physrev.00015.2009>
  79. Wambolt RB, Lopaschuk GD, Brownsey RW, Allard MF (2000) Dichloroacetate improves postischemic function of hypertrophied rat hearts. *J Am Coll Cardiol* 36(4):1378–1385
  80. Wambolt RB, Henning SL, English DR, Dyachkova Y, Lopaschuk GD, Allard MF (1999) Glucose utilization and glycogen turnover are accelerated in hypertrophied rat hearts during severe low-flow ischemia. *J Mol Cell Cardiol* 31(3):493–502. <https://doi.org/10.1006/jmcc.1998.0804>
  81. Ellis JM, Mentock SM, Depettrillo MA, Koves TR, Sen S, Watkins SM, Muoio DM, Cline GW, Taegtmeier H, Shulman GI, Willis MS, Coleman RA (2011) Mouse cardiac acyl coenzyme a synthetase 1 deficiency impairs fatty acid oxidation and induces cardiac hypertrophy. *Mol Cell Biol* 31(6):1252–1262. <https://doi.org/10.1128/MCB.01085-10>
  82. Hunter WG, Kelly JP, McGarrah RW, 3rd, Khouri MG, Craig D, Haynes C, Ilkayeva O, Stevens RD, Bain JR, Muehlbauer MJ, Newgard CB, Felker GM, Hernandez AF, Velazquez EJ, Kraus WE, Shah SH (2016) Metabolomic profiling identifies novel circulating biomarkers of mitochondrial dysfunction differentially elevated in heart failure with preserved versus reduced ejection fraction: evidence for shared metabolic impairments in clinical heart failure. *Journal of the American Heart Association* 5 (8). <https://doi.org/10.1161/JAHA.115.003190>
  83. Choi YS, de Mattos AB, Shao D, Li T, Nabben M, Kim M, Wang W, Tian R, Kolwicz SC Jr (2016) Preservation of myocardial fatty acid oxidation prevents diastolic dysfunction in mice subjected to angiotensin II infusion. *J Mol Cell Cardiol* 100:64–71. <https://doi.org/10.1016/j.yjmcc.2016.09.001>
  84. Dong Z, Zhao P, Xu M, Zhang C, Guo W, Chen H, Tian J, Wei H, Lu R, Cao T (2017) Astragaloside IV alleviates heart failure via activating PPARalpha to switch glycolysis to fatty acid beta-oxidation. *Scientific reports* 7(1):2691. <https://doi.org/10.1038/s41598-017-02360-5>
  85. Harmancey R, Vasquez HG, Guthrie PH, Taegtmeier H (2013) Decreased long-chain fatty acid oxidation impairs postischemic recovery of the insulin-resistant rat heart. *FASEB journal : Official publication of the Federation of American Societies for Experimental Biology* 27(10):3966–3978. <https://doi.org/10.1096/fj.13-234914>
  86. Pascual F (1861) Coleman RA (2016) Fuel availability and fate in cardiac metabolism: A tale of two substrates. *Biochem Biophys Acta* 10:1425–1433. <https://doi.org/10.1016/j.bbali.2016.03.014>
  87. Bing RJ, Siegel A, Ungar I, Gilbert M (1954) Metabolism of the human heart. *The American Journal of Medicine* 16(4):504–515. [https://doi.org/10.1016/0002-9343\(54\)90365-4](https://doi.org/10.1016/0002-9343(54)90365-4)
  88. Bedi KC Jr, Snyder NW, Brandimarto J, Aziz M, Mesaros C, Worth AJ, Wang LL, Javaheri A, Blair IA, Margulies KB, Rame JE (2016) Evidence for intramyocardial disruption of lipid metabolism and increased myocardial ketone utilization in advanced human heart failure. *Circulation* 133(8):706–716. <https://doi.org/10.1161/CIRCULATIONAHA.115.017545>
  89. Aubert G, Martin OJ, Horton JL, Lai L, Vega RB, Leone TC, Koves T, Gardell SJ, Kruger M, Hoppel CL, Lewandowski ED, Crawford PA, Muoio DM, Kelly DP (2016) The failing heart relies on ketone bodies as a fuel. *Circulation* 133(8):698–705. <https://doi.org/10.1161/CIRCULATIONAHA.115.017355>
  90. Huynh K (2016) Heart failure: ketone bodies as fuel in heart failure. *Nature reviews Cardiology* 13(3):122–123. <https://doi.org/10.1038/nrcardio.2016.22>
  91. Schugar RC, Moll AR, Andre d'Avignon D, Weinheimer CJ, Kovacs A, Crawford PA (2014) Cardiomyocyte-specific deficiency of ketone body metabolism promotes accelerated pathological remodeling. *Molecular metabolism* 3(7):754–769. <https://doi.org/10.1016/j.molmet.2014.07.010>
  92. Cook GA, Lavrentyev EN, Pham K (1861) Park EA (2017) Streptozotocin diabetes increases mRNA expression of ketogenic enzymes in the rat heart. *Biochim Biophys Acta* 2:307–312. <https://doi.org/10.1016/j.bbagen.2016.11.012>
  93. Fitchett D, Inzucchi SE, Cannon CP, McGuire DK, Scirica BM, Johansen OE, Sambevski S, Kaspers S, Pfarr E, George JT, Zinman B (2019) Empagliflozin reduced mortality and hospitalization for heart failure across the spectrum of cardiovascular risk in the EMPA-REG OUTCOME trial. *Circulation* 139(11):1384–1395. <https://doi.org/10.1161/CIRCULATIONAHA.118.037778>
  94. Ferrannini E, Mark M, Mayoux E (2016) CV protection in the EMPA-REG OUTCOME trial: A “thrifty substrate” hypothesis. *Diabetes Care* 39(7):1108–1114. <https://doi.org/10.2337/dc16-0330>
  95. Poulsen SH, Sogaard P, Nielsen-Kudsk JE, Egeblad H (2007) Recovery of left ventricular systolic longitudinal strain after valve replacement in aortic stenosis and relation to natriuretic peptides. *J Am Soc Echocardiogr* 20(7):877–884. <https://doi.org/10.1016/j.echo.2006.11.020>
  96. Khuchua ZA, Ventura-Clapier R, Kuznetsov AV, Grishin MN, Saks VA (1989) Alterations in the creatine kinase system in the myocardium of cardiomyopathic hamsters. *Biochem Biophys Res Commun* 165(2):748–757
  97. Nascimben L, Friedrich J, Liao R, Pauletto P, Pessina AC, Ingwall JS (1995) Enalapril treatment increases cardiac performance and energy reserve via the creatine kinase reaction in myocardium of Syrian myopathic hamsters with advanced heart failure. *Circulation* 91(6):1824–1833
  98. Neubauer S, Horn M, Naumann A, Tian R, Hu K, Laser M, Friedrich J, Gaudron P, Schnackerz K, Ingwall JS et al (1995) Impairment of energy metabolism in intact residual myocardium of rat hearts with chronic myocardial infarction. *J Clin Investig* 95(3):1092–1100. <https://doi.org/10.1172/jci117756>
  99. Tian R, Nascimben L, Ingwall JS, Lorell BH (1997) Failure to maintain a low ADP concentration impairs diastolic function in hypertrophied rat hearts. *Circulation* 96(4):1313–1319
  100. Smith CS, Bottomley PA, Schulman SP, Gerstenblith G, Weiss RG (2006) Altered creatine kinase adenosine triphosphate kinetics in failing hypertrophied human myocardium. *Circulation* 114(11):1151–1158. <https://doi.org/10.1161/circulationaha.106.613646>
  101. Kennel PJ, Mancini DM, Schulze PC (2015) Skeletal muscle changes in chronic cardiac disease and failure. *Comprehensive Physiology* 5(4):1947–1969. <https://doi.org/10.1002/cphy.c110003>
  102. Wu F, Zhang J, Beard DA (2009) Experimentally observed phenomena on cardiac energetics in heart failure emerge from simulations of cardiac metabolism. *Proc Natl Acad Sci USA* 106(17):7143–7148. <https://doi.org/10.1073/pnas.0812768106>
  103. 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 (2015) Synergistic role of ADP and Ca(2+) in diastolic myocardial stiffness. *The Journal of physiology* 593(17):3899–3916. <https://doi.org/10.1113/jp270354>
  104. Sequeira V, Najafi A, Wijnker PJ, Dos Remedios CG (2015) ADP-stimulated contraction: A predictor of thin-filament

- activation in cardiac disease 112(50):E7003-7012. <https://doi.org/10.1073/pnas.1513843112>
105. Chen Y, Zhang Z, Hu F, Yang W, Yuan J, Cui J, Hao S, Hu J, Zhou Y, Qiao S (2015) 17beta-estradiol prevents cardiac diastolic dysfunction by stimulating mitochondrial function: a preclinical study in a mouse model of a human hypertrophic cardiomyopathy mutation. *J Steroid Biochem Mol Biol* 147:92–102. <https://doi.org/10.1016/j.jsbmb.2014.12.011>
  106. Hiebert JB, Shen Q, Thimmesch A, Pierce J (2016) Impaired myocardial bioenergetics in HFpEF and the role of anti-oxidants. *Open Cardiovasc Med J* 10:158–162. <https://doi.org/10.2174/1874192401610010158>
  107. Silberman GA, Fan TH, Liu H, Jiao Z, Xiao HD, Lovelock JD, Boulden BM, Widder J, Fredd S, Bernstein KE, Wolska BM, Dikalov S, Harrison DG, Dudley SC Jr (2010) Uncoupled cardiac nitric oxide synthase mediates diastolic dysfunction. *Circulation* 121(4):519–528. <https://doi.org/10.1161/CIRCULATIONAHA.109.883777>
  108. Moslehi J, DePinho RA, Sahin E (2012) Telomeres and mitochondria in the aging heart. *Circ Res* 110(9):1226–1237. <https://doi.org/10.1161/CIRCRESAHA.111.246868>
  109. Birks EJ (2013) Molecular changes after left ventricular assist device support for heart failure. *Circ Res* 113(6):777–791. <https://doi.org/10.1161/circresaha.113.301413>
  110. Gruson D, Ahn SA, Rousseau MF (2011) Biomarkers of inflammation and cardiac remodeling: the quest of relevant companions for the risk stratification of heart failure patients is still ongoing. *Biochemia medica* 21(3):254–263
  111. Schwartz A, Lee KS (1962) Study of heart mitochondria and glycolytic metabolism in experimentally induced cardiac failure. *Circ Res* 10:321–332
  112. Bugger H, Schwarzer M, Chen D, Schreppler A, Amorim PA, Schoepe M, Nguyen TD, Mohr FW, Khalimonchuk O, Weimer BC, Doent T (2010) Proteomic remodelling of mitochondrial oxidative pathways in pressure overload-induced heart failure. *Cardiovasc Res* 85(2):376–384. <https://doi.org/10.1093/cvr/cvp344>
  113. Song M, Mihara K, Chen Y, Scorrano L, Dorn GW 2nd (2015) Mitochondrial fission and fusion factors reciprocally orchestrate mitophagic culling in mouse hearts and cultured fibroblasts. *Cell Metab* 21(2):273–286. <https://doi.org/10.1016/j.cmet.2014.12.011>
  114. Hoppel CL, Tandler B, Fujioka H, Riva A (2009) Dynamic organization of mitochondria in human heart and in myocardial disease. *The international journal of biochemistry & cell biology* 41(10):1949–1956. <https://doi.org/10.1016/j.biocel.2009.05.004>
  115. ES W, DW B, DE F (1986) Subpopulations of human heart mitochondria. *J Surg Res* 40 (5)
  116. Hollander JM, Thapa D, Shepherd DL (2014) Physiological and structural differences in spatially distinct subpopulations of cardiac mitochondria: Influence of cardiac pathologies. *Am J Physiol Heart Circ Physiol* 307(1):H1-14. <https://doi.org/10.1152/ajpheart.00747.2013>
  117. Schwarzer M, Schreppler A, Amorim PA, Osterholt M, Doent T (2013) Pressure overload differentially affects respiratory capacity in interfibrillar and subsarcolemmal mitochondria. *Am J Physiol Heart Circ Physiol* 304(4):H529-537. <https://doi.org/10.1152/ajpheart.00699.2012>
  118. Dabkowski ER, Williamson CL, Bukowski VC, Chapman RS, Leonard SS, Peer CJ, Callery PS, Hollander JM (2009) Diabetic cardiomyopathy-associated dysfunction in spatially distinct mitochondrial subpopulations. *Am J Physiol Heart Circ Physiol* 296(2):H359-369. <https://doi.org/10.1152/ajpheart.00467.2008>
  119. Dabkowski ER, Baseler WA, Williamson CL, Powell M, Razunguzwa TT, Frisbee JC, Hollander JM (2010) Mitochondrial dysfunction in the type 2 diabetic heart is associated with alterations in spatially distinct mitochondrial proteomes. *Am J Physiol Heart Circ Physiol* 299(2):H529-540. <https://doi.org/10.1152/ajpheart.00267.2010>
  120. Karamanlidis G, Nascimben L, Couper GS, Shekar PS, del Monte F, Tian R (2010) Defective DNA replication impairs mitochondrial biogenesis in human failing hearts. *Circ Res* 106(9):1541–1548. <https://doi.org/10.1161/CIRCRESAHA.109.212753>
  121. Padrao AI, Ferreira RM, Vitorino R, Alves RM, Neuparth MJ, Duarte JA (1807) Amado F (2011) OXPHOS susceptibility to oxidative modifications: the role of heart mitochondrial subcellular location. *Biochim Biophys Acta* 9:1106–1113. <https://doi.org/10.1016/j.bbabi.2011.04.002>
  122. Piquereau J, Caffin F, Novotova M, Lemaire C, Veksler V, Garnier A, Ventura-Clapier R, Joubert F (2013) Mitochondrial dynamics in the adult cardiomyocytes: which roles for a highly specialized cell? *Front Physiol* 4:102. <https://doi.org/10.3389/fphys.2013.00102>
  123. Jornayvaz FR, Shulman GI (2010) Regulation of mitochondrial biogenesis. *Essays Biochem* 47:69–84. <https://doi.org/10.1042/bse0470069>
  124. Bayeva M, Gheorghide M, Ardehali H (2013) Mitochondria as a therapeutic target in heart failure. *J Am Coll Cardiol* 61(6):599–610. <https://doi.org/10.1016/j.jacc.2012.08.1021>
  125. Pisano A, Cerbelli B, Perli E, Pelullo M, Bargelli V, Prezioso C, Mancini M, He L, Bates MG, Lucena JR, Della Monica PL, Familiari G, Petrozza V, Nediani C, Taylor RW, d'Amati G, Giordano C (2016) Impaired mitochondrial biogenesis is a common feature to myocardial hypertrophy and end-stage ischemic heart failure. *Cardiovascular pathology : The official journal of the Society for Cardiovascular Pathology* 25(2):103–112. <https://doi.org/10.1016/j.carpath.2015.09.009>
  126. Matliba MA, Rembert JC, Millarda RW, Ashrafa M, Rouslina W, Asano G Jr, JCG, Schwartz A, (1983) Mitochondrial function in canine experimental cardiac hypertrophy. *J Mol Cell Cardiol* 14(4):221–232. [https://doi.org/10.1016/0022-2828\(83\)90277-8](https://doi.org/10.1016/0022-2828(83)90277-8)
  127. Zhang X, Li ZL, Eirin A, Ebrahimi B, Pawar AS, Zhu XY, Lerman A, Lerman LO (2015) Cardiac metabolic alterations in hypertensive obese pigs. *Hypertension* 66(2):430–436. <https://doi.org/10.1161/HYPERTENSIONAHA.115.05478>
  128. Dai DF, Johnson SC, Villarin JJ, Chin MT, Nieves-Cintrón M, Chen T, Marcinek DJ, Dorn GW 2nd, Kang YJ, Prolla TA, Santana LF, Rabinovitch PS (2011) Mitochondrial oxidative stress mediates angiotensin II-induced cardiac hypertrophy and Galphaq overexpression-induced heart failure. *Circ Res* 108(7):837–846. <https://doi.org/10.1161/circresaha.110.232306>
  129. Chaanine AH, Joyce LD, Stulak JM, Maltais S, Joyce DL, Dearani JA, Klaus K, Nair KS, Hajjar RJ, Redfield MM (2019) Mitochondrial morphology, dynamics, and function in human pressure overload or ischemic heart disease with preserved or reduced ejection fraction. *Circulation Heart failure* 12(2):e005131. <https://doi.org/10.1161/CIRCHEARTFAILURE.118.005131>
  130. Chaanine AH, Kohlbrenner E, Gamb SI, Guenzel AJ, Klaus K, Fayyaz AU, Nair KS, Hajjar RJ, Redfield MM (2016) FOXO3a regulates BNIP3 and modulates mitochondrial calcium, dynamics, and function in cardiac stress. *American journal of physiology Heart and circulatory physiology* 311(6):H1540–H1559. <https://doi.org/10.1152/ajpheart.00549.2016>
  131. Lewis MR, Lewis WH (1914) Mitochondria in tissue culture. *Science* 39:330–333
  132. JM S, J. N (2002) Mitochondrial dynamics and division in budding yeast. *Trends Cell Biol* 12 (4)
  133. Twig G, Hyde B, Shirihai OS (2008) Mitochondrial fusion, fission and autophagy as a quality control axis: The bioenergetic view. *Biochim Biophys Acta* 1777(9):1092–1097. <https://doi.org/10.1016/j.bbabi.2008.05.001>

134. Chen KH, Guo X, Ma D, Guo Y, Li Q, Yang D, Li P, Qiu X, Wen S, Xiao RP, Tang J (2004) Dysregulation of HSG triggers vascular proliferative disorders. *Nat Cell Biol* 6(9):872–883. <https://doi.org/10.1038/ncb1161>
135. Konstantinidis K, Lederer WJ, Rizzuto R, Kitsis RN (2012) Mitofusin 2 joins the sarcoplasmic reticulum and mitochondria at the hip to sustain cardiac energetics. *Circ Res* 111(7):821–823. <https://doi.org/10.1161/CIRCRESAHA.112.277269>
136. Ong SB, Kalkhoran SB, Hernandez-Resendiz S, Samangouei P, Ong SG, Hausenloy DJ (2017) Mitochondrial-shaping proteins in cardiac health and disease—the long and the short of it! *Cardiovasc Drugs Ther* 31(1):87–107. <https://doi.org/10.1007/s10557-016-6710-1>
137. Brown DA, Perry JB, Allen ME, Sabbah HN, Stauffer BL, Shaikh SR, Cleland JG, Colucci WS, Butler J, Voors AA, Anker SD, Pitt B, Pieske B, Filippatos G, Greene SJ, Gheorghiadu M (2017) Expert consensus document: Mitochondrial function as a therapeutic target in heart failure. *Nature reviews Cardiology* 14(4):238–250. <https://doi.org/10.1038/nrcardio.2016.203>
138. Bhattacharya D, Mukhopadhyay M, Bhattacharyya M, Karmakar P (2018) Is autophagy associated with diabetes mellitus and its complications? A review. *EXCLI journal* 17:709–720. <https://doi.org/10.17179/excli2018-1353>
139. Tong M, Saito T, Zhai P, Oka SI, Mizushima W, Nakamura M, Ikeda S, Shirakabe A, Sadoshima J (2019) Mitophagy is essential for maintaining cardiac function during high fat diet-induced diabetic cardiomyopathy. *Circ Res* 124(9):1360–1371. <https://doi.org/10.1161/CIRCRESAHA.118.314607>
140. Che Y, Wang ZP, Yuan Y, Zhang N, Jin YG, Wan CX, Tang QZ (2018) Role of autophagy in a model of obesity: a long-term high fat diet induces cardiac dysfunction. *Molecular medicine reports* 18(3):3251–3261. <https://doi.org/10.3892/mmr.2018.9301>
141. Zhang K (2018) “NO” to autophagy: fat does the trick for diabetes. *Diabetes* 67(2):180–181. <https://doi.org/10.2337/dbi17-0048>
142. Ouyang C, You J, Xie Z (2014) The interplay between autophagy and apoptosis in the diabetic heart. *J Mol Cell Cardiol* 71:71–80. <https://doi.org/10.1016/j.yjmcc.2013.10.014>
143. Das A, Durrant D, Koka S, Salloum FN, Xi L, Kukreja RC (2014) Mammalian target of rapamycin (mTOR) inhibition with rapamycin improves cardiac function in type 2 diabetic mice: Potential role of attenuated oxidative stress and altered contractile protein expression. *The Journal of biological chemistry* 289(7):4145–4160. <https://doi.org/10.1074/jbc.M113.521062>
144. Hsu HC, Liu CH, Tsai YC, Li SJ, Chen CY, Chu CH, Chen MF (2016) Time-dependent cellular response in the liver and heart in a dietary-induced obese mouse model: The potential role of ER stress and autophagy. *Eur J Nutr* 55(6):2031–2043. <https://doi.org/10.1007/s00394-015-1017-8>
145. Bernhardt D, Muller M, Reichert AS, Osiewicz HD (2015) Simultaneous impairment of mitochondrial fission and fusion reduces mitophagy and shortens replicative lifespan. *Scientific reports* 5:7885. <https://doi.org/10.1038/srep07885>
146. Liem DA, Nsair A, Setty SP, Cadeiras M, Wang D, Maclellan R, Lotz C, Lin AJ, Tabaraki J, Li H, Ge J, Odeberg J, Ponten F, Larson E, Mulder J, Lundberg E, Weiss JN, Uhlen M, Ping P, Deng MC (2014) Molecular- and organelle-based predictive paradigm underlying recovery by left ventricular assist device support. *Circulation Heart failure* 7(2):359–366. <https://doi.org/10.1161/CIRCHEARTFAILURE.113.000250>
147. Zorova LD, Popkov VA, Plotnikov EY, Silachev DN, Pevzner IB, Jankauskas SS, Babenko VA, Zorov SD, Balakireva AV, Juhaszova M, Sollott SJ, Zorov DB (2018) Mitochondrial membrane potential. *Anal Biochem* 552:50–59. <https://doi.org/10.1016/j.ab.2017.07.009>
148. Sanbe A, Tanonaka K, Kobayashi R, Takeo S (1995) Effects of long-term therapy with ACE inhibitors, captopril, enalapril andtrandolapril, on myocardial energy metabolism in rats with heart failure following myocardial infarction. *J Mol Cell Cardiol* 27(10):2209–2222
149. Matlib MA, Rembert JC, Millard RW, Ashraf M, Rouslin W, Asano G, Greenfield JC Jr, Schwartz A (1983) Mitochondrial function in canine experimental cardiac hypertrophy. *J Mol Cell Cardiol* 15(4):221–232
150. Divakaruni AS, Brand MD (2011) The regulation and physiology of mitochondrial proton leak. *Physiology (Bethesda, Md)* 26(3):192–205. <https://doi.org/10.1152/physiol.00046.2010>
151. Eghtay KS (2007) Mitochondrial uncoupling proteins—what is their physiological role? *Free Radical Biol Med* 43(10):1351–1371. <https://doi.org/10.1016/j.freeradbiomed.2007.08.011>
152. Cadenas S (2018) Mitochondrial uncoupling, ROS generation and cardioprotection. *Biochim Biophys Acta* 1859(9):940–950. <https://doi.org/10.1016/j.bbabi.2018.05.019>
153. Zhao RZ, Jiang S, Zhang L, Yu ZB (2019) Mitochondrial electron transport chain, ROS generation and uncoupling (Review). *Int J Mol Med* 44(1):3–15. <https://doi.org/10.3892/ijmm.2019.4188>
154. Murray AJ, Cole MA, Lygate CA, Carr CA, Stuckey DJ, Little SE, Neubauer S, Clarke K (2008) Increased mitochondrial uncoupling proteins, respiratory uncoupling and decreased efficiency in the chronically infarcted rat heart. *J Mol Cell Cardiol* 44(4):694–700. <https://doi.org/10.1016/j.yjmcc.2008.01.008>
155. A N-S, C H, G C, R C, M T, R S, L P, L C (1997) A role for uncoupling protein-2 as a regulator of mitochondrial hydrogen peroxide generation. *FASEB J* 11 (10)
156. Teshima Y, Akao M, Jones SP, Marban E (2003) Uncoupling protein-2 overexpression inhibits mitochondrial death pathway in cardiomyocytes. *Circ Res* 93(3):192–200. <https://doi.org/10.1161/01.RES.0000085581.60197.4D>
157. Yang K, Xu X, Nie L, Xiao T, Guan X, He T, Yu Y, Liu L, Huang Y, Zhang J, Zhao J (2015) Indoxyl sulfate induces oxidative stress and hypertrophy in cardiomyocytes by inhibiting the AMPK/UCP2 signaling pathway. *Toxicol Lett* 234(2):110–119. <https://doi.org/10.1016/j.toxlet.2015.01.021>
158. Aon MA, Cortassa S, O'Rourke B (2010) Redox-optimized ROS balance: A unifying hypothesis. *Biochim Biophys Acta* 1797(6–7):865–877. <https://doi.org/10.1016/j.bbabi.2010.02.016>
159. Nickel AG, von Hardenberg A, Hohl M, Loffler JR, Kohlhaas M, Becker J, Reil JC, Kazakov A, Bonnekoh J, Stadelmaier M, Puhl S, Wagner M, Bogeski I, Cortassa S, Kappl R, Pasieka B, Lafontaine M, Lancaster CR, Blacker TS, Hall AR, Duchon MR, Kastner L, Lipp P, Zeller T, Muller C, Knopp A, Laufs U, Bohm M, Hoth M, Maack C (2015) Reversal of mitochondrial transhydrogenase causes oxidative stress in heart failure. *Cell Metab* 22(3):472–484. <https://doi.org/10.1016/j.cmet.2015.07.008>
160. Paulus WJ, Tschope C (2013) 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* 62(4):263–271. <https://doi.org/10.1016/j.jacc.2013.02.092>
161. Zeng H, Chen JX (2019) Microvascular rarefaction and heart failure with preserved ejection fraction. *Front Cardiovasc Med* 6:15. <https://doi.org/10.3389/fcvm.2019.00015>
162. Franssen C, Chen S, Unger A, Korkmaz HI, De Keulenaer GW, Tschope C, Leite-Moreira AF, Musters R, Niessen HW, Linke WA, Paulus WJ, Hamdani N (2016) Myocardial microvascular inflammatory endothelial activation in heart failure with preserved ejection fraction. *JACC Heart failure* 4(4):312–324. <https://doi.org/10.1016/j.jchf.2015.10.007>
163. Saiki H, Moulay G, Guenzel AJ, Liu W, Decklever TD, Classic KL, Pham L, Chen HH, Burnett JC, Russell SJ, Redfield MM (2017) Experimental cardiac radiation exposure induces



- ventricular diastolic dysfunction with preserved ejection fraction. *Am J Physiol Heart Circ Physiol* 313(2):H392–H407. <https://doi.org/10.1152/ajpheart.00124.2017>
164. Ide T, Tsutsui H, Hayashidani S, Kang D, Suematsu N, Nakamura K, Utsumi H, Hamasaki N, Takeshita A (2001) Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction. *Circ Res* 88(5):529–535
  165. Kohr MJ, Davis JP, Ziolo MT (2009) Peroxynitrite increases protein phosphatase activity and promotes the interaction of phospholamban with protein phosphatase 2a in the myocardium. *Nitric oxide : Biology and chemistry* 20(3):217–221. <https://doi.org/10.1016/j.niox.2009.01.003>
  166. Pinti MV, Hathaway QA, Hollander JM (2017) Role of micro-RNA in metabolic shift during heart failure. *Am J Physiol Heart Circ Physiol* 312(1):H33–H45. <https://doi.org/10.1152/ajpheart.00341.2016>
  167. Negi SI, Jeong EM, Shukrullah I, Veleder E, Jones DP, Fan TH, Varadarajan S, Danilov SM, Fukai T, Dudley SC Jr (2015) Renin-angiotensin activation and oxidative stress in early heart failure with preserved ejection fraction. *Biomed Res Int* 2015:825027. <https://doi.org/10.1155/2015/825027>
  168. Brown GC, Borutaite V (2007) Nitric oxide and mitochondrial respiration in the heart. *Cardiovasc Res* 75(2):283–290. <https://doi.org/10.1016/j.cardiores.2007.03.022>
  169. Mital S, Loke KE, Addonizio LJ, Oz MC, Hintze TH (2000) Left ventricular assist device implantation augments nitric oxide dependent control of mitochondrial respiration in failing human hearts. *J Am Coll Cardiol* 36(6):1897–1902
  170. Brown GC, Borutaite V (2002) Nitric oxide inhibition of mitochondrial respiration and its role in cell death. *Free Radical Biol Med* 33(11):1440–1450
  171. Brown GC (1999) Nitric oxide and mitochondrial respiration. *Biochem Biophys Acta* 1411(2–3):351–369
  172. Brookes PS, Zhang J, Dai L, Zhou F, Parks DA, Darley-Usmar VM, Anderson PG (2001) Increased sensitivity of mitochondrial respiration to inhibition by nitric oxide in cardiac hypertrophy. *J Mol Cell Cardiol* 33(1):69–82. <https://doi.org/10.1006/jmcc.2000.1276>
  173. Mihaylova MM, Shaw RJ (2011) The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nat Cell Biol* 13(9):1016–1023. <https://doi.org/10.1038/ncb2329>
  174. Takimoto E (2012) Cyclic GMP-dependent signaling in cardiac myocytes. *Circulation journal : Official journal of the Japanese Circulation Society* 76(8):1819–1825
  175. 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 (2013) Myocardial titin hypophosphorylation importantly contributes to heart failure with preserved ejection fraction in a rat metabolic risk model. *Circulation Heart failure* 6(6):1239–1249. <https://doi.org/10.1161/CIRCHEARTFAILURE.113.000539>
  176. Grutzner A, Garcia-Manyes S, Kotter S, Badilla CL, Fernandez JM, Linke WA (2009) Modulation of titin-based stiffness by disulfide bonding in the cardiac titin N2-B unique sequence. *Biophys J* 97(3):825–834. <https://doi.org/10.1016/j.bpj.2009.05.037>
  177. Paulus WJ, Vantrimpont PJ, Shah AM (1994) Acute effects of nitric oxide on left ventricular relaxation and diastolic distensibility in humans. Assessment by bicoronary sodium nitroprusside infusion. *Circulation* 89 (5):2070–2078
  178. Layland J, Li JM, Shah AM (2002) Role of cyclic GMP-dependent protein kinase in the contractile response to exogenous nitric oxide in rat cardiac myocytes. *The Journal of physiology* 540(Pt 2):457–467
  179. Szelenyi Z, Fazakas A, Szenasi G, Kiss M, Tegze N, Fekete BC, Nagy E, Bodo I, Nagy B, Molvarec A, Patocs A, Pepo L, Prohaszka Z, Vereckei A (2015) Inflammation and oxidative stress caused by nitric oxide synthase uncoupling might lead to left ventricular diastolic and systolic dysfunction in patients with hypertension. *Journal of geriatric cardiology : JGC* 12(1):1–10. <https://doi.org/10.11909/j.issn.1671-5411.2015.01.001>
  180. van Empel V, Brunner-La Rocca HP (2015) Inflammation in HFpEF: Key or circumstantial? *Int J Cardiol* 189:259–263. <https://doi.org/10.1016/j.ijcard.2015.04.110>
  181. Farrar EJ, Huntley GD, Butcher J (2015) Endothelial-derived oxidative stress drives myofibroblastic activation and calcification of the aortic valve. *PLoS one* 10(4):e0123257. <https://doi.org/10.1371/journal.pone.0123257>
  182. Zhao W, Zhao T, Chen Y, Ahokas RA, Sun Y (2008) Oxidative stress mediates cardiac fibrosis by enhancing transforming growth factor-beta1 in hypertensive rats. *Mol Cell Biochem* 317(1–2):43–50. <https://doi.org/10.1007/s11010-008-9803-8>
  183. Kuwahara F, Kai H, Tokuda K, Kai M, Takeshita A, Egashira K, Imaizumi T (2002) Transforming growth factor-beta function blocking prevents myocardial fibrosis and diastolic dysfunction in pressure-overloaded rats. *Circulation* 106(1):130–135
  184. Kasner M, Westermann D, Lopez B, Gaub R, Escher F, Kuhl U, Schultheiss HP, Tschope C (2011) Diastolic tissue Doppler indexes correlate with the degree of collagen expression and cross-linking in heart failure and normal ejection fraction. *J Am Coll Cardiol* 57(8):977–985. <https://doi.org/10.1016/j.jacc.2010.10.024>
  185. Rodrigues PG, Leite-Moreira AF, Falcao-Pires I (2016) Myocardial reverse remodeling: how far can we rewind? 310 (11):H1402–1422. <https://doi.org/10.1152/ajpheart.00696.2015>
  186. Sharma K, Kass DA (2014) Heart failure with preserved ejection fraction: Mechanisms, clinical features, and therapies. *Circ Res* 115(1):79–96. <https://doi.org/10.1161/circresaha.115.302922>
  187. Ahmed SH, Clark LL, Pennington WR, Webb CS, Bonnema DD, Leonardi AH, McClure CD, Spinale FG, Zile MR (2006) Matrix metalloproteinases/tissue inhibitors of metalloproteinases: Relationship between changes in proteolytic determinants of matrix composition and structural, functional, and clinical manifestations of hypertensive heart disease. *Circulation* 113(17):2089–2096. <https://doi.org/10.1161/circulationaha.105.573865>
  188. Fukuhara E, Yamada T, Morita T, Furukawa Y, Tamaki S, Iwasaki Y, Kawasaki M, Kikuchi A, Kawai T, Seo M, Ikeda I, Abe M, Nakamura J, Kayama K, Kawahira M, Tanabe K, Fukunami M (2017) Prognostic value of diuretic resistance in patients admitted for acute decompensated heart failure with reduced and preserved left ventricular ejection fraction: a prospective study. *Circulation* 136 (S1)
  189. Reddy YNV, Obokata M, Koepp KE, Egbe AC, Wiley B, Borlaug BA (2019) The beta-adrenergic agonist albuterol improves pulmonary vascular reserve in heart failure with preserved ejection fraction. *Circ Res* 124(2):306–314. <https://doi.org/10.1161/CIRCRESAHA.118.313832>
  190. Andersen MJ, Hwang SJ, Kane GC, Melenovsky V, Olson TP, Fetterly K, Borlaug BA (2015) Enhanced pulmonary vasodilator reserve and abnormal right ventricular: Pulmonary artery coupling in heart failure with preserved ejection fraction. *Circulation Heart failure* 8(3):542–550. <https://doi.org/10.1161/CIRCHEARTFAILURE.114.002114>
  191. Yamamoto K (2017) Pharmacological treatment of heart failure with preserved ejection fraction. *Yonago Acta Medica* 60:71–76
  192. Adeniran I, MacIver DH, Hancox JC, Zhang H (2015) Abnormal calcium homeostasis in heart failure with preserved ejection fraction is related to both reduced contractile function and incomplete relaxation: an electromechanically detailed biophysical modeling study. *Front Physiol* 6:78. <https://doi.org/10.3389/fphys.2015.00078>



193. Santulli G, Xie W, Reiken SR, Marks AR (2015) Mitochondrial calcium overload is a key determinant in heart failure. *Proc Natl Acad Sci USA* 112(36):11389–11394. <https://doi.org/10.1073/pnas.1513047112>
194. Hasenfuss G, Maier LS (2008) Mechanism of action of the new anti-ischemia drug ranolazine. *Clinical research in cardiology : Official journal of the German Cardiac Society* 97(4):222–226. <https://doi.org/10.1007/s00392-007-0612-y>
195. Fraser H, Belardinelli L, Wang L, Light PE, McVeigh JJ, Clanachan AS (2006) Ranolazine decreases diastolic calcium accumulation caused by ATX-II or ischemia in rat hearts. *J Mol Cell Cardiol* 41(6):1031–1038. <https://doi.org/10.1016/j.yjmcc.2006.08.012>
196. Dietl A, Maack C (2017) Targeting mitochondrial calcium handling and reactive oxygen species in heart failure. *Current heart failure reports* 14(4):338–349. <https://doi.org/10.1007/s11897-017-0347-7>
197. Heggermont WA, Papageorgiou AP, Heymans S, van Bilsen M (2016) Metabolic support for the heart: Complementary therapy for heart failure? *Eur J Heart Fail* 18(12):1420–1429. <https://doi.org/10.1002/ejhf.678>
198. Gevaert AB, Boen JRA, Segers VF, Van Craenenbroeck EM (2019) Heart failure with preserved ejection fraction: a review of cardiac and noncardiac pathophysiology. *Frontiers in physiology* 10:638. <https://doi.org/10.3389/fphys.2019.00638>
199. Lund LH, Claggett B, Liu J, Lam CS, Jhund PS, Rosano GM, Swedberg K, Yusuf S, Granger CB, Pfeffer MA, McMurray JJV, Solomon SD (2018) Heart failure with mid-range ejection fraction in CHARM: Characteristics, outcomes and effect of candesartan across the entire ejection fraction spectrum. *Eur J Heart Fail* 20(8):1230–1239. <https://doi.org/10.1002/ejhf.1149>
200. Ding W, Liu T, Bi X, Zhang Z (2017) Mitochondria-targeted antioxidant mito-tempo protects against aldosterone-induced renal injury in vivo. *Cellular physiology and biochemistry : International journal of experimental cellular physiology, biochemistry, and pharmacology* 44(2):741–750. <https://doi.org/10.1159/000485287>
201. Hirsch GA, Bottomley PA, Gerstenblith G, Weiss RG (2012) Allopurinol acutely increases adenosine triphosphate energy delivery in failing human hearts. *J Am Coll Cardiol* 59(9):802–808. <https://doi.org/10.1016/j.jacc.2011.10.895>
202. Cappola TP, Kass DA, Nelson GS, Berger RD, Rosas GO, Kobeissi ZA, Marban E, Hare JM (2001) Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy. *Circulation* 104(20):2407–2411. <https://doi.org/10.1161/hc4501.098928>
203. Ribeiro Junior RF, Dabkowski ER, Shekar KC, KA OC, Hecker PA, Murphy MP, (2018) MitoQ improves mitochondrial dysfunction in heart failure induced by pressure overload. *Free Radical Biol Med* 117:18–29. <https://doi.org/10.1016/j.freeradbiomed.2018.01.012>
204. Chirinos JA, Zamani P (2016) The nitrate-nitrite-NO pathway and its implications for heart failure and preserved ejection fraction. *Current heart failure reports* 13(1):47–59. <https://doi.org/10.1007/s11897-016-0277-9>
205. Maier LS, Layug B, Karwatowska-Prokopczuk E, Belardinelli L, Lee S, Sander J, Lang C, Wachter R, Edelmann F, Hasenfuss G, Jacobshagen C (2013) RAnoLazIne for the treatment of diastolic heart failure in patients with preserved ejection fraction: the RALIDHF proof-of-concept study. *JACC Heart failure* 1(2):115–122. <https://doi.org/10.1016/j.jchf.2012.12.002>
206. Lopatin Y (2016) Trimetazidine in the new 2016 European guidelines on heart failure and beyond. *Heart Metab* 71:23–26
207. Piao L, Fang YH, Cadete VJ, Wietholt C, Urboniene D, Toth PT, Marsboom G, Zhang HJ, Haber I, Rehman J, Lopaschuk GD, Archer SL (2010) The inhibition of pyruvate dehydrogenase kinase improves impaired cardiac function and electrical remodeling in two models of right ventricular hypertrophy: Resuscitating the hibernating right ventricle. *J Mol Med* 88(1):47–60. <https://doi.org/10.1007/s00109-009-0524-6>
208. Zhao G, Jeoung NH, Burgess SC, Rosaaen-Stowe KA, Inagaki T, Latif S, Shelton JM, McAnally J, Bassel-Duby R, Harris RA, Richardson JA, Kliewer SA (2008) Overexpression of pyruvate dehydrogenase kinase 4 in heart perturbs metabolism and exacerbates calcineurin-induced cardiomyopathy. *American journal of physiology Heart and circulatory physiology* 294(2):H936–943. <https://doi.org/10.1152/ajpheart.00870.2007>
209. Fragasso G, Salerno A, Spoladore R, Bassanelli G, Arioli F, Margonato A (2008) Metabolic therapy of heart failure. *Curr Pharm Des* 14(25):2582–2591. <https://doi.org/10.2174/138161208786071245>
210. Pierce JD, Mahoney DE, Hiebert JB, Thimmesch AR, Diaz FJ, Smith C, Shen Q, Mudaranthakam DP, Clancy RL (2018) Study protocol, randomized controlled trial: Reducing symptom burden in patients with heart failure with preserved ejection fraction using ubiquinol and/or D-ribose. *BMC cardiovascular disorders* 18(1):57. <https://doi.org/10.1186/s12872-018-0796-2>
211. Lam CSP, Voors AA, de Boer RA, Solomon SD, van Veldhuisen DJ (2018) Heart failure with preserved ejection fraction: From mechanisms to therapies. *Eur Heart J* 39(30):2780–2792. <https://doi.org/10.1093/eurheartj/ehy301>
212. Kitzman DW, Brubaker P, Morgan T, Haykowsky M, Hundley G, Kraus WE, Eggebeen J, Nicklas BJ (2016) 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* 315(1):36–46. <https://doi.org/10.1001/jama.2015.17346>
213. Carbone S, Mauro AG, Mezzaroma E, Kraskauskas D, Marchetti C, Buzzetti R, Van Tassell BW, Abbate A, Toldo S (2015) A high-sugar and high-fat diet impairs cardiac systolic and diastolic function in mice. *Int J Cardiol* 198:66–69. <https://doi.org/10.1016/j.ijcard.2015.06.136>
214. von Bibra H, Strohle A, St John Sutton M, Worm N (2017) Dietary therapy in heart failure with preserved ejection fraction and/or left ventricular diastolic dysfunction in patients with metabolic syndrome. *Int J Cardiol* 234:7–15. <https://doi.org/10.1016/j.ijcard.2017.01.003>
215. Carbone S, Canada JM, Buckley LF, Trankle CR, Billingsley HE, Dixon DL, Mauro AG, Dessie S, Kadariya D, Mezzaroma E, Buzzetti R, Arena R, Van Tassell BW, Toldo S, Abbate A (2017) Dietary fat, sugar consumption, and cardiorespiratory fitness in patients with heart failure with preserved ejection fraction. *JACC Basic to translational science* 2(5):513–525. <https://doi.org/10.1016/j.jacbts.2017.06.009>
216. Yan Y, Jiang W, Spinetti T, Tardivel A, Castillo R, Bourquin C, Guarda G, Tian Z, Tschopp J, Zhou R (2013) Omega-3 fatty acids prevent inflammation and metabolic disorder through inhibition of NLRP3 inflammasome activation. *Immunity* 38(6):1154–1163. <https://doi.org/10.1016/j.immuni.2013.05.015>
217. Wang DD, Li Y, Chiuve SE, Stampfer MJ, Manson JE, Rimm EB, Willett WC, Hu FB (2016) Association of specific dietary fats with total and cause-specific mortality. *JAMA internal medicine* 176(8):1134–1145. <https://doi.org/10.1001/jamainternmed.2016.2417>
218. Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, Gomez-Gracia E, Ruiz-Gutierrez V, Fiol M, Lapetra J, Lamuela-Raventos RM, Serra-Majem L, Pinto X, Basora J, Munoz MA, Sorli JV, Martinez JA, Martinez-Gonzalez MA, Investigators PS (2013) Primary prevention of cardiovascular disease with a Mediterranean diet. *The New England journal of medicine* 368(14):1279–1290. <https://doi.org/10.1056/NEJMoa1200303>
219. Dearlove DJ, Faull OK, Clarke K (2019) Context is key: Exogenous ketosis and athletic performance. *Current Opinion in Physiology* 10:81–89. <https://doi.org/10.1016/j.cophys.2019.04.010>

220. Sharp TE 3rd, Lefler DJ, Houser SR (2019) Cardiometabolic heart failure and HFpEF: Still chasing unicorns. *JACC Basic to translational science* 4(3):422–424. <https://doi.org/10.1016/j.jacbts.2019.05.003>

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