Obesity is a Major Determinant of Impaired Cardiac Energy Metabolism in Heart Failure with Preserved Ejection Fraction

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ABBREVIATIONS

CPT-1b: Carnitine-palmitoyl transferase 1b
EF: Ejection fraction
ERK: Extracellular signal-regulated kinase
EDP: End-diastolic pressure
ESP: End-systolic pressure
FS: Fractional shortening
GLS: Global longitudinal strain
HFD: High-fat diet
HFpEF: Heart failure with preserved ejection fraction
HFrEF: Heart failure with reduced ejection fraction
HRP: Horseradish peroxidase
HW: Heart weight
ipGTT: Intraperitoneal glucose tolerance test
L-NAME: L-NG-Nitro arginine methyl ester
LV: Left ventricle
LW: Lung weight
NLRP3: NLR family pyrin domain containing 3
PDH: Pyruvate dehydrogenase
PGC1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PPARα: Peroxisome proliferator-activated receptor α
PV: Pressure-Volume
PVDF: Polyvinylidene fluoride
SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TBS-T: Tris-buffered saline containing Tween 20
TFAM: Mitochondrial transcription factor A
TL: Tibia length
βOHb: β-hydroxybutyrate
ABSTRACT

Heart failure with preserved ejection fraction (HFpEF) is a major health problem with limited treatment options. Although optimizing cardiac energy metabolism is a potential approach to treating heart failure, it is poorly understood what alterations in cardiac energy metabolism actually occur in HFpEF. To determine this, we used mice in which HFpEF was induced using an obesity and hypertension HFpEF protocol for 10 weeks. Next, carvedilol, a third-generation β-blocker and a biased agonist that exhibits agonist-like effects through β arrestins by activating extracellular signal-regulated kinase (ERK), was used to decrease one of these parameters, namely hypertension. Heart function was evaluated by invasive pressure-volume loops and echocardiography, as well as by ex vivo working heart perfusions. Glycolysis and oxidation rates of glucose, fatty acids, and ketones were measured in the isolated working hearts. The development of HFpEF was associated with a dramatic decrease in cardiac glucose oxidation rates, with a parallel increase in palmitate oxidation rates. Carvedilol treatment decreased the development of HFpEF, but had no major effect on cardiac energy substrate metabolism. Carvedilol treatment did increase the expression of cardiac β arrestin 2 and proteins involved in mitochondrial biogenesis. Decreasing body weight in obese HFpEF mice increased glucose oxidation and improved heart function. This suggests that the dramatic energy metabolic changes in HFpEF mice hearts are primarily due to the obesity component of the HFpEF model.

Key Words: HFpEF, glucose oxidation, fatty acid oxidation, carvedilol, β arrestin 2
SIGNIFICANCE STATEMENT

- Metabolic inflexibility occurs in HFP EF mice hearts
- Lowering blood pressure improves heart function in HFP EF mice with no major effect on energy metabolism
- Between hypertension and obesity, the latter appears to have the major role in HFP EF cardiac energetic changes
- Carvedilol increases mitochondrial biogenesis and overall energy expenditure in HFP EF hearts
INTRODUCTION

Heart failure with preserved ejection fraction (HFpEF) is a worldwide public health problem that affects more than 64 million people (Collaborators, 2018). Common comorbidities related with HFpEF include other cardiovascular diseases such as hypertension, atrial fibrillation, and metabolic disorders such as diabetes and obesity. Myocardial stiffening, fibrosis and diastolic dysfunction play central pathophysiological roles in HFpEF (Paulus and Tschöpe, 2013).

In contrast to heart failure with reduced ejection fraction (HFrEF), there are limited treatments for patients with HFpEF. Thus, the treatment of HFpEF remains challenging. Beta blockers are one of the main group of drugs used for the treatment of HFrEF (Cleland et al., 2017; Tsutsui et al., 2019). They are also recommended for some cases of HFpEF, although their efficacy for this indication is still under debate (Members et al., 2012; Bavishi et al., 2015; Xu and Wang, 2020). Clinical studies reported no benefit (Hernandez et al., 2009), deteriorated (Farasat et al., 2010) or improved (Liu et al., 2014; Lund et al., 2014) prognosis of β blocker treatment in patients with HFpEF. Potential benefits of high doses of carvedilol in HFpEF patients have been proposed to be beneficial in HFpEF (Yamamoto et al., 2013; Lam et al., 2018). Notebly, carvedilol and bisoprolol have been shown to reduce mortality and morbidity in HFpEF patients with new-onset diabetes (Garcia-Egido et al., 2015), and to improve prognosis in HFpEF (Ruiz et al., 2016).
Carvedilol is a third generation β blocker that is used for the treatment of heart failure, hypertension, and for the prevention of ventricular dysfunction that can develop after myocardial infarction. It is one of the few β antagonists used in heart failure accompanied by metabolic disorders such as insulin resistance or diabetes (Pollare et al., 1989; Lithell et al., 1992; Jacob et al., 1996). In addition, it has been recognized as a biased agonist due to the fact that it shows its effect through β arrestins (Wisler et al., 2007). Carvedilol activates extracellular signal-regulated kinase (ERK) via β arrestin, while blocking Gs-dependent adenylate cyclase activation. Little is known about physiological relevance and the signaling process regulated by β arrestins. However, there seems to be a strong correlation between β arrestin 1 and/or 2 deficiency, diabetes and cardiovascular diseases (Luan et al., 2009; Bathgate-Siryk et al., 2014).

Several studies suggest that HFpEF stems from the convergence of two major comorbidities seen in HFpEF patients: mechanical loading, and metabolic stress such as obesity (Schiattarella et al., 2019). Hence, the two-hit hypothesis (metabolic stress and hypertension) has been claimed as a major mechanism that underlies HFpEF pathophysiology (Roh et al., 2022). Of importance, both obesity and hypertension can adversely affect cardiac energy metabolism, particularly glucose oxidation (Sankaralingam et al., 2014; Karwi et al., 2019). We have previously demonstrated that lowering body weight in obese mice with heart failure was associated with decreased cardiac hypertrophy and improvements in both cardiac insulin sensitive glucose oxidation and diastolic function (Sankaralingam et al., 2014; Karwi et al., 2019). We therefore hypothesized that in HFpEF the primary energy metabolic change is a decrease in glucose oxidation, and that the obesity component of HFpEF is the major
determinant of the cardiac metabolic changes. In this study we aimed to define the contribution of hypertension versus obesity to the potential deterioration of cardiac energy metabolism in HFpEF mice. To do this, we used carvedilol to reduce mechanical load in obese and hypertensive mice and asked two questions: 1) What are the cardiac energy metabolic changes that occur in HFpEF mice?, and 2) What effect does reducing hypertension with carvedilol in HFpEF mice have on cardiac function and energy metabolism?
MATERIALS AND METHODS

**Animals:** Animals have been handled in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health. All procedures and the care of mice were approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee (AUP-288), and conformed to the guidelines of the Canadian Council on Animal Care. Mice were housed at the University of Alberta Health Sciences Laboratory Animal Services facility in a temperature- and humidity- controlled room with a 12 hour (h) light-dark cycle.

**Experimental Groups:** Fifty-four, 8-week-old male C57Bl/6J mice (Charles River Laboratories, Wilmington, MA, USA) were randomly divided into 3 groups:

1) Controls (n=18),

2) HFpEF mice: High-fat diet (Research Diets Inc, catalog#D12492 HFD, 60% fat) + L-NAME (Sigma-Aldrich catalog#N5751, 0.5 g/L) (n=18),

3) HFpEF mice+Carvedilol: High-fat diet [Research Diets Inc, costumized, catalog#D21102703-5 HFD, 60% fat, (the same ingredients with #D12492+carvedilol)] + L-NAME (0.5 g/L)+carvedilol (30 mg/kg) (Nguyen et al., 2019) (n=18).

Control mice were fed with a regular chow diet (PicoLab catalog#5L0D, 5% fat) while HFpEF groups were fed with a HFD and L-NAME in the drinking water for 10 weeks. Carvedilol (Santa Cruz, catalog# sc-200157) was added to HFD food pellets by homogenously mixing the drug with the chow. Food (Supplemental Figure 1A) and water (Supplemental Figure 1B) consumption levels of the animals were followed on a
daily basis. At week 9 of the HFD treatment, mice were subjected to an intraperitoneal glucose tolerance test (ipGTT, 2g/kg glucose).

Exploratory evaluations of the heart function and energy metabolism were completed by using echocardiography, in-vivo pressure-volume measurements and isolated working heart perfusions.

To assess the effects of weight loss on cardiac function and energy metabolism, two additional groups of animals were studied in which HFpEF was produced in mice with an abdominal aortic constriction and a HFD, followed by switching to a low fat diet (LFD) to decrease body weight. 8-week-old C57Bl/6J mice were fed a HFD for 4 weeks, following which they were anesthetised with 0.75% isoflurane and underwent either a sham or abdominal aortic constriction surgery, as described previously (Zhang et al., 2013). Mice were kept on the HFD for a further 4 weeks and then randomly divided into mice that were continued on a HFD for an additional 10 weeks, or mice that were switched to a LFD for a further 10 weeks:

1. HFpEF: HFD
2. HFpEF+LFD: HFD was changed to a low fat diet (5% fat).

Figure 1A and Figure 5A summarize the experimental timelines of the study.

**Echocardiography:** At week 10 of treatment, transthoracic echocardiography was performed using a small animal imaging ultrasound system to evaluate cardiac function and wall thickness (Vevo 3100 high-resolution imaging system equipped with a 30-MHz transducer, MX550S; VisualSonics, Toronto, Canada). All mice were anesthetized with 1.5% isoflurane. Wall measurements, left ventricular mass, diastolic and diastolic...
function, heart rate, tissue Doppler parameters, ejection fraction (%EF) and fractional shortening (%FS) were measured using m-mode taken from parasternal long-axis and short-axis views at the mid-papillary level. Left ventricular dimensions were measured during both systole and diastole. Pressure gradient (mmHg) was calculated from the mitral flow using the velocity–time integral measurement. All measurements were performed in a blinded fashion.

**Pressure-Volume (PV) Loop Analysis:** To measure the left ventricle (LV) pressure-volume relationship, mice were anesthetized with isoflurane. An incision was made in the right carotid artery and a 1.2F admittance catheter (Scisense Inc.) was inserted into the incision. The catheter was advanced to the LV via the ascending aorta and aortic valve. The position of the catheter was monitored by pressure, along with the magnitude and phase using an ADvantage pressure-volume system (Scisense Inc.) and a data acquisition system (iWorx Systems Inc.) connected to the catheter. A baseline scan was performed to derive volume, using Baan's equation, and a pressure-volume loop was obtained using the LabScribe2 software (version 2.347000). At the final stage, the inferior vena cava was briefly occluded to obtain changes in venous returns to derive end-systolic and end-diastolic PV relations. Online and offline calculations were performed using LabScribe2 software (Version 2.347000). All measurements were performed in a blinded fashion.

**Isolated Working Heart Perfusions:** Energy metabolism in hearts was evaluated by performing isolated working heart perfusions. Mice were initially anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg). Hearts were then excised
and perfused for 60 min with Krebs–Henseleit solution containing 2.5 mM Ca^{2+}, 5 mM glucose, 0.8 mM palmitate (pre-bound to 3% bovine serum albumin, BSA), and 0.6 mM β-hydroxybutyrate (βOHB). Insulin was added to perfusion buffer at the 30th min of the protocol to evaluate a possible effect of this intervention.

Glycolysis, glucose oxidation, ketone oxidation and palmitate oxidation rates were measured by sampling $^{14}$CO$_2$ and $^3$H$_2$O produced from the metabolism of [5-$^3$H] glucose, [U-$^{14}$C] glucose, [3-$^{14}$C] βOHB, and [9,10-$^3$H] palmitate, respectively. For ATP production, glycolysis, glucose oxidation, ketone oxidation and palmitate oxidation rates were multiplied by the number of ATP molecules produced from each process (2, 29, 22 and 105, respectively). The results were converted into percentages to compare the contribution of each substrate to total cardiac ATP production. All measurements were performed in a blinded fashion.

At the end of the perfusion, hearts were snap-frozen with liquid nitrogen and stored at -80°C until processed for further analysis.

**Western Blots:** Frozen whole heart tissues were homogenized in RIPA buffer. The protein concentrations of tissue lysates were measured using the Bradford protein assay. Equal amounts of protein were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After transferring proteins to a nitrocellulose or polyvinylidene fluoride (PVDF) membrane overnight at 22V, membranes were incubated with tris-buffered saline containing 0.1% Tween 20 (TBS-T) and 5% milk powder for 1 h at room temperature for blocking. Membranes were then incubated
overnight at 4°C with the following primary antibodies: β arrestin 1 (Cell Signaling Technology, catalog#30036, RRID: AB_2798985, 1:1000), β arrestin 2 (Cell Signaling Technology, catalog#3857, RRID: AB_225868, 1:1000), phospho-Akt (Cell Signaling Technology, catalog#9271S, AB_329825, 1:500), Akt (Cell Signaling Technology, catalog#9272S, RRID: AB_329827, 1:1000), phospho-pyruvate dehydrogenase (phospho-PDH) (Cell Signaling Technology, catalog#31866, RRID: AB_2799014, 1:1000), PDH (Cell Signaling Technology, catalog#2784, RRID: AB_2162928, 1:1000), phospho-ERK (Cell Signaling Technology, catalog#9101, RRID: AB_331646, 1:2000), ERK (Cell Signaling Technology, catalog#9102, RRID: AB_330744, 1:2000), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α, Santa Cruz, catalog#sc13067, RRID: AB_2166218, 1:500), phospho-PPARα (p-PPARα, Abcam, catalog#ab3484, RRID: AB_303844, 1:1000), carnitine-palmitoyl transferase 1b (CPT-1b, Novus Biologicals catalog#NBP2-92666, RRID: AB_2924792, 1:500), NLR family pyrin domain containing 3 (NLRP3, Cell Signaling Technology, catalog#15101, RRID: AB_2722591, 1:1000), Sirtuin1 (Sirt1, Abcam, catalog#ab110304, RRID: AB_10864359, 1:1000), mitochondrial transcription factor A (TFAM, Santa Cruz, catalog#sc-376672, RRID: AB_11150497, 1:1000), Citrate Synthase (Santa Cruz, catalog#sc-242444, RRID: AB_10848152, 1:1000), α tubulin (Cell Signaling Technology, catalog#3873, RRID: AB_1904178, 1:3000), vinculin (Thermofisher Scientific catalog#700062, RRID: AB_2532280, 1:3000). Membranes were washed for 30 min with TBS-T and incubated for 1 h at room temperature with horseradish peroxidase (HRP)-conjugated anti-rabbit (Cell Signaling Technology, catalog#7074, RRID: AB_2099233, 1:5000) or anti-mouse (BioRad, catalog#170-6516, RRID: AB_11125547, 1:5000) secondary antibodies. After further washing, membranes were incubated with enhanced
chemiluminescence for 2 min; and chemiluminescent signals were developed on X-ray film. Some membranes were stripped after the incubation with an antibody before they were used for another one. Quantification of the signals was performed with Image J®.

**Statistical Analysis:** Data are presented throughout as the mean ± SEM. Significant differences were determined by using One-Way ANOVA or Two-Way ANOVA followed by a Tukey post hoc test. Statistical analysis were carried out using commercially available software (GraphPad Prism V9). Differences were deemed statistically significant when p<0.05.

Further information related to Supplemental Methods is provided in Supplemental Data.

**RESULTS**

**Animal Characteristics:** Body weights of the animals fed the HFD were higher than controls starting by the second week of HFD treatment (Figure 1B). At the end of week 9, mice were subjected to an ipGTT. Blood glucose levels of HFpEF groups were significantly higher than those of controls at nearly all time points (Figure 1C).

To evaluate the effect of the HFpEF model on the weights of the heart and lungs, we calculated the heart weight to tibia length ratio (HW/TL) and lung weight (LW) to tibia length ratio (LW/TL) after we sacrificed the animals. A higher HW/TL ratio was observed only in untreated HFpEF group (Figure 1D). LW/TL were the same in all groups (Figure 1E). HW, TL and LW (wet/dry) values were also presented seperately (Supplemental Figure 2A, 2B and 2C, respectively).
Carvedilol Improves Cardiac Function in HFpEF: Figure 2 shows the noninvasive echocardiography results and in vivo PV loop analysis of the animals. LV mass slightly increased in the HFpEF groups (Figure 2A). As expected, ejection fraction of the hearts were preserved in the HFpEF groups (Figure 2B). E/A (Figure 2C) and E/E’ (Figure 2D) ratios were slightly higher in HFpEF mice. By lowering blood pressure and thus loading, carvedilol treatment improved global longitudinal strain (GLS), which is a marker of LV systolic function (Figure 2E).

Both carvedilol-treated and untreated HFpEF animals had higher end-systolic pressure (ESP) than controls (Figure 2F). Carvedilol prevented the increase in end-diastolic pressure (EDP) (Figure 2G). Further, carvedilol improved Tau Mirsky when compared to untreated HFpEF animals (Figure 2H). Figure 2I and Figure 2J are representative images of PV Loop and echocardiography, respectively. Additional echocardiographic and in vivo cardiac parameters are summarized in Supplemental Table1 and Supplemental Table2, respectively.

In the isolated working hearts we measured cardiac work, oxygen consumption and cardiac efficiency. Compared to controls, cardiac work was lower in the HFpEF group. The decrease in cardiac work was prevented by carvedilol (Figure 3A and 3B). Additionally, carvedilol prevented the drop in oxygen consumption observed in HFpEF (Figure 3C and 3D). Cardiac efficiency was similar in all groups except for a slight decline in the untreated HFpEF group (Figure 3E and 3F). Additional functional parameters obtained during heart perfusions are shown in Supplemental Table3.
Energy Substrate Utilization Was Altered in HFpEF Mice: To evaluate energy substrate metabolism of the hearts, glucose, palmitate and βOHB utilization rates were assessed. In the absence of insulin, higher rates of myocardial glycolysis were observed in carvedilol-treated HFpEF mice (Figure 4A). Interestingly, addition of insulin to the perfusate did not affect the rates of glycolysis. On the other hand, the rates of glucose oxidation were lower in HFpEF hearts compared to controls. Addition of insulin dramatically stimulated glucose oxidation in control hearts only (Figure 4B). βOHB oxidation was slightly higher in controls (Figure 4C). Palmitate oxidation rates were significantly higher in untreated HFpEF hearts compared to controls in the presence of insulin. Independent of the presence of insulin, rates of palmitate oxidation were higher in carvedilol treated-HFpEF hearts (Figure 4D). However, when we normalized the metabolic data to unit of cardiac work (Supplemental Figure 3), palmitate oxidation rates decreased in carvedilol-treated hearts compared to untreated hearts.

ATP production rates of the hearts were evaluated by calculating the ATP production from the metabolism of each substrate. Total ATP production of carvedilol-treated hearts was higher than both control and untreated HFpEF groups (Figure 4E). When compared to controls, palmitate oxidation had a higher contribution to the overall ATP production in the HFpEF groups (Figure 4F).

Carvedilol Increases β Arrestin 2 and Mitochondrial Biogenesis Proteins in HFpEF Hearts: Beta arrestin protein expression and ERK phosphorylation were examined to understand the contribution of biased agonism to the effects of carvedilol. Beta arrestin 1 was not affected by HFD or carvedilol treatment (Figure 5A) while β arrestin 2 levels
were increased with carvedilol treatment (Figure 5B). Chronic treatment with carvedilol did not change ERK phosphorylation (Figure 5C, Supplemental Figure 4A).

Insulin phosphorylates PPARα at serine 12 through ERK (Burns and Vanden Heuvel, 2007). Therefore, serine 12 phosphorylation status of PPARα was assessed. Carvedilol treatment increased PPARα phosphorylation at this site (Figure 6A). In line with the increase in palmitate oxidation rates, CPT1β levels were higher in both untreated and carvedilol-treated HFpEF hearts compared to controls (Figure 6B). PDH phosphorylation was similar in all groups (Figure 6C, Supplemental Figure 4C). To understand the effects of carvedilol on Akt phosphorylation, and thereby insulin signaling, phospho-Akt expression was evaluated. Interestingly, carvedilol stimulated Akt phosphorylation in HFpEF hearts (Figure 6D, Supplemental Figure 4B).

As it is the transcriptional coactivator of both mitochondrial biogenesis and PPARα (a regulator of fatty acid metabolism), PGC1α protein expression was evaluated. Carvedilol treatment increased the expression of PGC1α (Figure 7A). Next, the expression of Sirt1 were investigated for its role in deacetylation of PGC1α. Sirt1 expression was increased with carvedilol treatment (Figure 7B). As a downstream target of PGC1α for mitochondrial biogenesis, we then evaluated TFAM expression. A higher TFAM expression was observed in carvedilol-treated hearts.

Citrate synthase is involved in mitochondrial oxidation process and accepted as a mitochondrial content biomarker (Larsen et al., 2012). Thus, we next investigated citrate
synthase levels and found that they were higher in both untreated and carvedilol-treated HFpEF hearts (Figure 7D).

**Switching to a Low Fat Diet Improved Metabolic Alterations in HFpEF Hearts:** After 8 weeks, the studies were continued in 2 groups of HFpEF mice by either switching mice to a LFD from a HFD, or keeping mice on a HFD during the next 10 weeks (Figure 8A). At the end of 18 weeks, the HFpEF+LFD group had lower body weight as expected (Figure 8B). Ejection fraction was preserved (Figure 8C) and diastolic dysfunction was improved, as evidenced by the E'/A' ratio (Figure 8D). Switching to a LFD provided the heart with a better insulin response. Glucose oxidation was increased by insulin administration in HFpEF+LFD hearts (Figure 8E), whereas palmitate oxidation did not change (Figure 8F).
DISCUSSION

Despite being a major health problem, there are few therapies that have been proven to be effective in treating HFpEF. In this study, we demonstrate that a dramatic alteration in energy substrate use was observed in HFpEF mice, particularly a decrease in glucose oxidation and an increase in fatty acid oxidation. The HFpEF model was generated by using a 2-hit (obesity and hypertension) protocol (Schiattarella et al., 2019). Body weights as well as blood pressure, both systolic and diastolic, were higher in HFpEF mice. Insulin resistance in HFpEF mice was shown by ipGTT. Reducing mechanical load in hypertensive mice by carvedilol partly removed one of the “hits” and improved cardiac function. However, carvedilol had no major effect on energy substrate metabolism, suggesting that the major contributor to the cardiac energy metabolic changes in HFpEF was obesity. This is further supported by comparing the direct effect of body weight reduction on both heart function and energy substrate metabolism, where we kept one group of mice on a HFD and switched the other group to a LFD. The decrease in body weight in the latter group was accompanied by improved glucose oxidation.

Based on the study by Schiattarella et al (26), we initially examined the mice by echocardiography on the sixth week of HFD treatment with L-NAME in the drinking water. We did not observe the expected deterioration of cardiac function (results not shown) and therefore extended the protocol for additional 4 weeks. At this time frame, a higher HW/TL ratio in the HFpEF groups indicated cardiac hypertrophy. In addition to the in vivo data showing a deterioration (note higher ESP and EDP values of HFpEFs), the E/E’ ratio was slightly lower, but not significant in the HFpEF groups. A potential
explanation for the lower E/E’ in HFpEF animals may be the rather young age of the male mouse, since some earlier studies reported a stronger HFpEF model by a 3-hit protocol (age, obesity and hypertension) (Withaar et al., 2021; Liu et al., 2022). To further confirm the establishment of the model (Shen et al., 2022), the levels of NLRP3, an inflammation marker, were evaluated in the whole heart. HFpEFs had higher levels of NLRP3 (Supplemental Figure 5). When combined, our data suggests the establishment of a mild HFpEF model that is mostly associated with obesity with the contribution of the increase in load.

Carvedilol improved both diastolic and systolic dysfunction in HFpEF animals as shown by an increase of EDP and GLS. In addition to lowering blood pressure, carvedilol ameliorated cardiac hypertrophy as evidenced by the similar ratios of HW/TL between control and carvedilol-treated HFpEF mice. Therefore, by decreasing hypertension, carvedilol could prevent the development of HFpEF.

Cardiac energy metabolism was significantly altered in HFpEF. We demonstrate that the main metabolic shift seen in HFpEF is a dramatic decrease in insulin-stimulated glucose oxidation, resulting in an “energy starved heart”. We also demonstrate that the beneficial effects of carvedilol on cardiac function were not associated with major changes in cardiac energy metabolism. This suggests that the metabolic changes seen in HFpEF hearts are due primarily to the obesity component of the model. However, carvedilol did stimulate insulin-independent glucose use, as seen by higher rates of glycolysis in carvedilol-treated animals without insulin in the perfusate which. This is consistent with our previous study that, when added to C2C12 cells, carvedilol
stimulates glycolysis and reduces palmitate oxidation (Onay-Besikci et al., 2012). We also showed that acute administration of carvedilol stimulated ERK phosphorylation and explained this by the biased agonist effect of the drug (Guven et al., 2020). The present study confirms that chronic usage of carvedilol stimulates β arrestin 2 expression.

Despite the lack of energy metabolic changes, treatment with carvedilol appeared to have some beneficial effects on mitochondria. PGC-1α [a co-activator of PPARα and the initiator of mitochondrial biogenesis (Duncan et al., 2007)] was higher in carvedilol-treated hearts. In addition to stimulation of mitochondrial biogenesis, the increase in PGC-1α serves to stimulate fatty acid utilization by activating PPARα (Vega et al., 2000; Fukushima and Lopaschuk, 2016). This is reflected in higher rates of ATP production in carvedilol-treated HFpEF hearts.

Hyperacetylation of mitochondrial proteins in HFpEF has recently been reported by Liu et al (Liu et al., 2022). SIRT1 is an enzyme that mediates NAD+ -dependent deacetylation of target substrates. A direct interaction and activation of PGC-1α by SIRT1 has been documented (Rodgers et al., 2005). We analyzed SIRT1 expression in our model and showed that the increase in SIRT1 expression by carvedilol was accompanied by an increase of PGC-1α in our HFpEF mouse model. The increase in other mitochondrial proteins, namely TFAM and citrate synthase confirmed the stimulation of mitochondrial biogenesis with carvedilol treatment in HFpEFs. In addition, the increase of citrate synthase in untreated HFpEFs suggest that the model challenged the hearts to increase mitochondrial function perhaps through stimulation of fatty acid oxidation as discussed earlier.
LIMITATIONS

HFpEF is the result of numerous co-morbidities, with obesity and hypertension representing two of these co-morbidities. Joe Hill’s group recently described a mouse model of HFpEF that combines obesity and hypertension to produce clinically relevant symptoms of HFpEF in young male mice (Schiattarella et al., 2019); the model we used in this study. However, clinically HFpEF is often seen in older patients, and is prevalent in females. Therefore, ideally aged and female mice should also be considered in this model of HFpEF. Indeed, our ongoing studies are now using aged female mice subjected to the obesity and hypertension protocol.

A carvedilol-treated control group was not included since carvedilol has long been used as a drug with established effects in both humans and animals.—In this study we specifically added a carvedilol group in the HFpEF mice because we anticipated that it would lower blood pressure and not obesity.
CONFLICT OF INTEREST

None.

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DATA AVAILABILITY STATEMENT

The authors declare that all the data supporting the findings of this study are available within the paper and its Supplemental Data.

AUTHOR CONTRIBUTION

Participated in research design: Güven, Onay-Besikci and Lopaschuk

Conducted experiments: Güven, Sun, Wagg, Almeida de Oliveira, Silver, Persad, Vu

Performed data analysis: Güven

Wrote or contributed to the writing of the manuscript: Güven, Onay-Besikci, Oudit and Lopaschuk
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FIGURE LEGENDS

Figure 1. Experimental time line (A), body weight (B), ipGTT (C), HW/TL (D) and LW (E) of the animals. * p<0.05 vs control (n=17-18). Significant differences were determined by using One-Way ANOVA followed by a Tukey post hoc test (HW: heart weight; ipGTT: intraperitoneal glucose tolerance test; LW: lung weight; TL: tibia length;).

Figure 2. LV Mass (A), ejection fraction (B), E/A ratio (C), E/E’ ratio (D), GLS (E), ESP (F), EDP (G), Tau (Mirsky/Glantz) (H), representative images of PV Loop (I) and echocardiography (J) of the hearts (Echocardiography n=6-12; PV Loop n=4-8). Significant differences were determined by using One-Way ANOVA followed by a Tukey post hoc test (EDP: end diastolic pressure; ESP: end systolic pressure; GLS: global longitudinal strain;).

Figure 3. Average values of cardiac work (A), oxygen consumption (C), cardiac efficiency (E); changes in cardiac work (B), oxygen consumption (D), cardiac efficiency (F) during the entire protocol obtained from isolated working heart perfusion (n=6-12). Significant differences were determined by using One-Way ANOVA followed by a Tukey post hoc test.

Figure 4. Glycolysis (A), glucose oxidation (B), βOHB oxidation (C) palmitate oxidation (D), and ATP production rates (E) of the hearts, and the percent contribution of each substrate to ATP production (F) * p<0.05 vs corresponding control (n=4-10). Significant differences were determined by using One-Way or Two-Way ANOVA followed by a Tukey post hoc test (ATP: adenosine triphosphate; βOHB: β-hydroxybutyrate).
Figure 5. β arrestin 1 (A), β arrestin 2 (B) and p-ERK protein expression (C) of the hearts (n=6). All samples were derived at the same time and processed in parallel. Significant differences were determined by using One-Way ANOVA followed by a Tukey post hoc test (ERK: extracellular signal-regulated kinase).

Figure 6. phospho(Ser12)-PPARα (A), CPT1b (B), phospho-PDH (C) and phospho(Ser473)-Akt (D) expression levels of the hearts. Values were normalized to α tubulin or the total amount of corresponding protein (n=6). All samples were derived at the same time and processed in parallel. Significant differences were determined by using One-Way ANOVA followed by a Tukey post hoc test (CPT1b: Carnitine-palmitoyl transferase 1b; PDH: Pyruvate dehydrogenase; Peroxisome proliferator-activated receptor α)

Figure 7. PGC1α (A), Sirt1 (B), TFAM (C) and Citrate Synthase (D) expression levels of the hearts. Values were normalized to α tubulin or vinculin (n=6). All samples were derived at the same time and processed in parallel. Significant differences were determined by using One-Way ANOVA followed by a Tukey post hoc test (PGC1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; TFAM: Mitochondrial transcription factor A).

Figure 8. Experimental time line (A), body weight (B), ejection fraction (C), E'/A' (D), glucose oxidation (E), palmitate oxidation (F) of continued HFpEF and HFpEF+LFD groups (n=5-7). Significant differences were determined by using a Student t-test or Two-Way ANOVA followed by a Tukey post hoc test (LFD: Low fat diet).
A. 

Starting point

8 weeks old male C57BL6 mice

Control: Regular chow diet
HFPpEF: HFD + L-NAME
HFPpEF + Carvedilol: HFD + L-NAME + Carvedilol

B.

Body Weight (g)

Week

0 1 2 3 4 5 6 7 8 9 10

C.

Blood Glucose (mmol/L)

Minutes

0 15 30 45 60 75 90 105 120

D.

HW / TL (mg/mm)

Control  HFPpEF  HFPpEF + Carvedilol

E.

LW / TL (g/mm)

Control  HFPpEF  HFPpEF + Carvedilol

Figure 1
Figure 2
Figure 3

A. Cardiac Work (joules * min^-1 * g dry wt^-1)

B. Cardiac Work (joules * min^-1 * g dry wt^-1)

C. Oxygen Consumption (μmol * min^-1 * g dry wt^-1)

D. Oxygen Consumption (μmol * min^-1 * g dry wt^-1)

E. Cardiac Efficiency (mmHg * mL / μmol)

F. Cardiac Efficiency (joules / μmol)

- Control
- HFrEF
- HFrEF + Carvedilol
- Insulin
- +Insulin

- Insulin
- +Insulin

- Insulin
- +Insulin

- Insulin
- +Insulin

- Insulin
- +Insulin
Figure 4
Figure 5
Figure 6