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Freshly isolated mitochondria from failing human hearts exhibit preserved respiratory function

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Abstract

In heart failure mitochondrial dysfunction is thought to be responsible for energy depletion and contractile dysfunction. The difficulties in procuring fresh left ventricular (LV) myocardium from humans for assessment of mitochondrial function have resulted in the reliance on surrogate markers of mitochondrial function and limited our understanding of cardiac energetics. We isolated mitochondria from fresh LV wall tissue of patients with heart failure and reduced systolic function undergoing heart transplant or left ventricular assist device placement, and compared their function to mitochondria isolated from the non-failing LV (NFLV) wall tissue with normal systolic function from patients with pulmonary hypertension undergoing heart-lung transplant. We performed detailed mitochondrial functional analyses using 4 substrates: glutamate-malate (GM), pyruvate-malate (PM) palmitoyl carnitine-malate (PC) and succinate. NFLV mitochondria showed preserved respiratory control ratios and electron chain integrity with only few differences for the 4 substrates. In contrast, HF mitochondria had greater respiration with GM, PM and PC substrates and higher electron chain capacity for PM than for PC. Surprisingly, HF mitochondria had greater respiratory control ratios and lower ADP-independent state 4 rates than NFLV mitochondria for GM, PM and PC substrates demonstrating that HF mitochondria are capable of coupled respiration

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ex vivo. Gene expression studies revealed decreased expression of key genes in pathways for oxidation of both fatty acids and glucose. Our results suggest that mitochondria from the failing LV myocardium are capable of tightly coupled respiration when isolated and supplied with ample substrates. Thus energy starvation in the failing heart may be the result of dysregulation of metabolic pathways, impaired substrate supply or reduced mitochondrial number but not the result of reduced mitochondrial electron transport capacity.

Keywords

Human heart failure; Mitochondrial function; Oxidative phosphorylation; Electron transport chain

1. Introduction

Our understanding of the energetic state of the heart in heart failure (HF) with reduced systolic function in humans is largely derived from animal studies which have documented impairments in mitochondrial respiration and myocardial energy metabolism [1, 2]. Specifically, reduced state 3 respiratory rates and respiratory control ratios (RCR) have been reported in HF [3]. The few published studies of failing human myocardial tissue seem concordant with the findings from animal studies [4–6]. However, most prior studies showing defects in mitochondrial electron transport chain (ETC) activity were based on functional assays in either left atrial appendages or permeabilized ventricular muscle bundles, or complex activity assays in frozen cardiac tissue or mitochondrial particles [7–10]. While these studies have given very valuable information regarding energetics of the failing myocardium, the functional integrity of isolated HF mitochondria, independent of the endogenous substrates, inhibitors and structural orientation, remains to be completely assessed. Further, investigation in atrial appendage tissue may not reflect mitochondrial functional changes in the left ventricular (LV) wall tissue of the heart. Given the difficulty in obtaining adequate amounts of fresh human LV wall tissue, published results from respiratory studies performed on mitochondrial isolates from fresh human ventricular wall tissue have been very few with contrasting results [11–14]. The time from procurement to processing, the tissue quantity, variability in sampling site (atrial appendage vs. ventricular wall tissue), and preparations of stripped fibers vs. isolates have compromised the implementation of integrative mitochondrial respiratory protocols. Further, in contrast to the large body of literature supporting mitochondrial dysfunction in HF, some studies [11, 14] have shown preserved mitochondrial functional capabilities in HF. Clearly, limitations on access of human cardiac tissue and variations in the techniques used to assess mitochondrial function have resulted in a lack of agreement regarding the energetics of the failing myocardium.

The aim of this study was to assess the impact of chronic HF on the functional integrity of mitochondria isolated from human left LV wall samples. Our objectives included the establishment of protocols for timely and consistent procurement and processing of failing human cardiac tissue for mitochondrial respiratory measurements in a reproducible manner; and the investigation of mitochondrial isolates independent of their *in situ* conditions for ETC capacity and oxidative phosphorylation.

2. Materials and Methods

2.1. Patient population

We isolated mitochondria from samples of LV apex cores from 24 patients undergoing left ventricular assist device (LVAD) placement, orthotopic heart transplantation, or heart-lung transplantation at the Houston Methodist Hospital. Of these 24 samples, 4 non-failing LV (NFLV) samples were obtained from patients undergoing heart-lung transplantation for pulmonary hypertension (PHTN) and served as comparators. PHTN patients had severe right ventricle failure with normal LV function assessed by echocardiography and histological analysis (Table 1, Fig. S1). We chose not to use the traditional control heart samples obtained from donor hearts unsuitable for transplantation because such samples are often exposed to a death-related catecholamine surge and are kept in cardioplegic solution for an indefinite period of time. There often is a long delay between the time of procurement and mitochondrial isolation. In addition, the use of cardioplegic solutions are reported to alter mitochondrial function [15]. However, these NFLV samples are not 'normal' or 'control' samples since they are undoubtedly altered by the systemic and pharmacological environment associated with severe right ventricular failure. Table 1 lists the demographic and clinical characteristics of the 24 patients used in this study. We analyzed samples based on etiology, body mass index (BMI), diabetes status and duration of HF. The study was approved by the Houston Methodist Hospital's institutional review board and all participants were enrolled only after signing informed consent.

2.2. Tissue procurement

Tissue procurement occurred in the operating room during orthotopic heart transplant, heart-lung transplant or LVAD placement procedures. After the heart or LV core tissue was handed over to us by the surgeon in the operating room, it was immediately transported to an adjacent laboratory. From the heart, 2 to 3 grams of LV tissue was excised at or near the apex. Then, approximately 200 mg of scar-free myocardium was dissected out and promptly placed into ice-cold buffer A (220 mmol/L mannitol, 70 mmol/L sucrose, and 5 mmol/L MOPS) [16]. The tissue was processed further for mitochondrial isolation. The time from surgeon hand-off until the initial homogenization step was less than 10 minutes.

2.3. Mitochondrial isolation

Mitochondria were isolated using a differential centrifugation method modified for small sample volumes from the protocol described by Frezza et al. [17]. Briefly, tissue was homogenized with a polytron homogenizer for two 5-second pulses, then centrifuged at 68 g at 4°C for 10 minutes. The supernatant fraction was collected and centrifuged at 18,000× g for 10 minutes. The resulting supernatant fraction was discarded but the pellet was re-suspended in cold buffer B (2 mmol/L EGTA, 0.2% fatty acid-free-BSA in buffer A [16]) and again centrifuged at 18,000× g for 10 minutes. The resulting pellet was rinsed with buffer A, centrifuged at the same speed for the same period, after which the pellet was re-suspended in 30 µl of cold buffer E (0.05 mmol/L EGTA in buffer A). The final mitochondrial volume was approximately 50 µl. This isolation technique results in a population of predominately subsarcolemmal mitochondria (SSM) with limited interfibrillar mitochondria (IFM) included in the isolate. For respiratory measurements, exactly 5 µl of

the mitochondrial suspension of approximately 20 ug/ul concentration or approximately 100 ug mitochondria were used in each assay. All readings were normalized to mg of mitochondrial protein after determination of the precise mitochondrial protein content by the Lowry assay (Biorad, Hercules, CA).

2.4. Mitochondrial respiration protocol

A high resolution Oroboros respirometer (Oroboros Instruments, Innsbruck, Austria) was used to assess oxygen consumption as a measurement of mitochondrial function. In each of the 2 chambers of the Oroboros respirometer, mitochondria were suspended in 2 ml respiration buffer consisting of EGTA (0.5 mmol/L), MgCl₂·6H₂O (3 mmol/L), K-lactobionate (60 mmol/L), Taurine (2 mmol/L), KH₂PO₄ (10 mmol/L), HEPES (20 mmol/L), sucrose (110 mmol/L), fatty acid free bovine serum albumin (1 g/L) at pH 7.1 (MiRO5 buffer). Oxygen consumption was studied in response to 4 substrates: palmitoylcarnitine-malate (PC, 5 mmol/L), pyruvate-malate (PM, 5 mmol/L), glutamate-malate (GM, 5 mmol/L) and succinate (Succinate, 5 mmol/L + Rotenone 0.1 μmol/L) with ADP (100 μmol/L), at 37°C. The sequence of addition was as follows: mitochondria—substrate (PC/P/G/Succinate)—ADP—malate (for PC/P/G)—FCCP (Fig. S2). Designations of respiration states followed those previously described by Chance and Williams [18], with state 1 respiration representing oxygen flux measured with mitochondria alone, state 2 with the addition of substrate to the mitochondrial isolate, state 3 after ADP addition and state 4 as ADP-limited respiration (Fig. S2). Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP, 2.0 μmol/L) was added after mitochondria reached state 4 for each substrate, to measure the uncoupled rate of respiration. The respiratory rates are reported as O₂ flux per mass (nmols/(min*mg)), and all readings are normalized to milligrams of protein in mitochondrial isolates. We chose to normalize to protein content rather than to citric synthase activity which can decrease after mitochondrial isolation and result in falsely elevated rates of respiration [19]. RCR was calculated as the ratio of state 3/state 4 rates, P/E ratio was calculated as the ratio of state 3/FCCP rates, and L/E ratio was calculated as state 4/FCCP rates.

2.5. RNA purification, cDNA synthesis and quantitative RT-PCR

Heart RNA was isolated using RNeasy kits (QIAGEN, Germantown, MD), reversed-transcribed into cDNA with High Capacity cDNA Reverse Transcription kits (Applied Biosystems, Grand Island, NY), and analyzed for candidate gene expression using Taqman PCR Core reagent kits and gene-specific primer/probe sets (Applied Biosystems). Candidate gene expression was normalized to *Ppia* (cyclophilin) expression [20].

2.6. Statistics

GraphPad Prism 5 was used for all statistical analyses. Mitochondrial respiration in NFLV and HF LV samples were compared by Mann-Whitney nonparametric analyses. A two-tailed $\alpha=0.05$ was used as the significance cut-off for all tests. Significance was defined as $P<0.05$, and sample sizes are indicated in each figure legend.

3. Results

3.1. Patient demographics

Of the 24 patients in our study, 18 were male and 15 were Caucasian (Table 1). Their average age was 57 ± 14 years. LV heart failure with reduced ejection fraction (HFrEF)[21], as seen by greatly decreased ejection fractions (EF, $19 \pm 10\%$) and enlarged left ventricles (LV internal diameter at diastole, LVIDd, 6.6 ± 0.8 cm), was present in 20 patients; whereas, 4 patients with PHTN had normal LV function, with normal EF and LVIDd. Further, NFLV samples from PHTN patients had no fibrosis while LV samples from HF patients had marked collagen staining indicative of massive fibrosis (Fig. S1). Of the 20 HF patients, 12 had ischemic cardiomyopathy (ICM) and 8 had non-ischemic cardiomyopathy (NICM). Subjects presented with several co-morbidities such as hypertension in 15 patients, dyslipidemia in 10, and type 2 diabetes in 10 patients. The average BMI was $28.6 \pm 7.4 \text{ kg/m}^2$ and the average duration of disease was 7 ± 5.6 years. All patients received HF medications that followed standard treatment American College of Cardiology/American Heart Association clinical practice guidelines [22, 23]. See Table 1 for medications, and demographic and clinical characteristics.

3.2. Assessment of mitochondrial respiration from human heart tissue

To determine the function of mitochondria isolated from human LV tissue samples, we assessed oxygen consumption in response to PC, PM, GM and succinate in presence of ADP. Mitochondria isolated from NFLV tissue samples had similar state 3 and state 4 respiration rates for all 4 substrates (Fig. 1A–B). However, RCR for GM was significantly higher than RCR for all other substrates (Fig. 1C). Uncoupled FCCP rates, a measurement of maximal ETC capacity, also did not differ for the 4 substrates in the NFLV mitochondria (Fig. 1D). The ratio of state 3 respiration to the uncoupled rate (P/E ratio, Fig. 1E), a measurement of phosphorylation control, and the ratio of state 4 respiration to uncoupled rate (L/E, Fig. 1F), a measurement of proton leak and any contaminating ATPases, were not different among the 4 substrates for NFLV. These results suggest that under ideal conditions, mitochondria isolated from human heart may have greater respiratory coupling with glutamate, but no overall differences between capacities to oxidize the different substrates *ex vivo*.

3.3. Mitochondria isolated from the LV wall of HF patients have preserved respiratory function

Unexpectedly, we found that mitochondria isolated from HF patients were capable of coupled oxidative phosphorylation for PC, PM and GM (Fig. 2). State 3 respiration rates of HF mitochondria did not differ for the 4 substrates (Fig. 2A), and were within the range of our comparator values. However, state 4 respiration rates for PC, PM or GM were markedly lower than those for succinate (Fig. 2B).

Correspondingly, the RCR for succinate was lower than RCR for all other substrates (Fig. 2C). RCR for GM was significantly greater than PC but unlike our comparator samples it was similar to that of PM. Again, RCR values for HF mitochondria for all substrates were within or greater than the range of previously reported state 3 and RCR values for cardiac

mitochondria in mouse models [3, 24, 25] or humans [11, 12, 14]. Uncoupled or maximal respiratory FCCP rates for GM were the lowest among FCCP rates for all substrates, and FCCP rate for PM was significantly greater than PC (Fig. 2D). Although the FCCP rates for PM and PC were dramatically different, the state 4 leak rates were similar for the two substrates. Corresponding to the FCCP rates, P/E ratios were the highest for GM, and P/E ratio for PM and succinate were similar, but lower than PC (Fig. 2E). L/E ratios were lower in PC and PM compared with Succinate and GM, and L/E ratio for PM was lower than that for PC, reflecting the relatively low uncoupled rate for PC (Fig. 2F). These data indicate that, although there are differences in the handling the 4 substrates, at least a subpopulation of mitochondria isolated from chronically failing human LV tissue possesses preserved ETC capacity and is capable of coupled respiration in response to various substrates.

3.4. Mitochondria isolated from LVs of HF patients exhibit higher respiratory function than mitochondria from NFLV tissue

Using the above data, we compared mitochondrial respiration of NFLV and HF by normalizing the HF results to NFLV results (Fig. 3). State 3 and state 4 rates were not significantly different between NFLV and HF mitochondria for the 4 substrates (Fig. 3A–B). However, RCRs for substrates PC, PM and GM were significantly greater in HF mitochondria than NFLV mitochondria (Fig. 3C), indicative of enhanced coupling between oxidation and phosphorylation for these complex I substrates in HF. FCCP rates (Fig. 3D) and P/E ratios (Fig. 3E) were similar in HF and NFLV mitochondria for all substrates. Whereas, L/E ratios were significantly lower in HF mitochondria when PC or PM were used (Fig. 3F). These results suggest that mitochondria from chronic HF LVs have enhanced respiratory capacity with reduced state 4 leak for complex I substrates than NFLV mitochondria.

3.5. Differential oxidation of complex I and complex II substrates by HF mitochondria

To determine whether mitochondria from HF or NFLV exhibit differential of NAD⁺-linked complex I and FAD-linked complex II substrates, we compared respiration rates for complex I substrates (PM, GM) and complex II substrate (succinate). PC is NAD⁺ and FAD-linked. In HF mitochondria, succinate-dependent respiration was highly uncoupled, indicated by high state 4 respiration and reduced RCR compared with all complex I substrates (Fig. 2B–C). Such complex II-specific difference in RCR or uncoupling was not evident in the NFLV mitochondria, although the complex I substrate GM appears to be the most efficiently oxidized substrate for this mitochondrial population (Fig. 1C). These data indicate that complex I-dependent PM and GM respiration is coupled and enhanced in HF, whereas complex II-dependent succinate respiration is associated with greater uncoupling.

3.6. Effects of etiology, diabetes, obesity and duration of disease on mitochondrial function

To determine whether etiology, co-morbidities and duration of HF affect mitochondrial respiratory capacity, we compared HF mitochondrial function in ICM vs. NICM, diabetic vs. non-diabetic, obese (BMI>30) vs. non-obese (BMI<30) and acute (<2 years) vs. chronic (>2 years) of HF (Fig. S3–6). While there were no differences between ICM vs. NICM in state 3, state 4, RCR, FCCP or P/E ratio for any of the substrates, L/E ratio was significantly

greater in ICM for Succinate (Fig. S3F). RCR for PC was significantly greater in LV mitochondria from obese patients compared with those from non-obese patients (Fig. S4C). In mitochondria from diabetic patients, state 3 rates were significantly lower for Succinate (Fig. S5A), while no other parameters were significantly affected by diabetes in HF. No differences were observed in mitochondrial respiration between HF patients with <2 years vs. >2 years of HF (Fig. S6). These results suggest that HF might induce an adaptive change in mitochondrial function, based on etiology, diabetes or obesity, where, ICM is associated with greater proton leak with Succinate, obesity is associated with greater mitochondrial respiration with PC, and diabetes is associated with a reduced state 3 respiration for Succinate.

3.7. Transcriptional changes in metabolic genes in HF

In order to determine regulation of substrate utilization in failing and non-failing hearts, we examined expression of key metabolic genes by measuring their transcript levels. Our previous study in human LV apex samples from failing and non-failing hearts showed that the failing heart reverts to a fetal metabolic gene profile by down-regulation of adult metabolic gene transcripts rather than by increasing fetal genes [26]. Consistent with our previous study, gene expression analyses of NFLV and HF samples showed increased expression of fatty acid uptake gene CD36 in HF, however, all fatty acid oxidation pathway genes, including CPT2, HADHA, ACADvl, ACADl, ACADm, PPAR α , AMPK and PGC1 α were down-regulated in HF (Fig. 4A–B). In contrast to previous animal studies of HF [27], expression of the uncoupling protein UCP3 was not significantly different in HF vs. NFLV samples (Fig S7). Similarly, most genes of the glucose and pyruvate metabolism pathways, including PDHB, ME3, GLUT1 were also decreased in expression in HF, suggesting that HF is associated with a global downregulation of adult metabolic gene pathways *in vivo*.

4. Discussion

The failing human heart is believed to be “energy-starved” due to compromised mitochondrial function [1]. Our results provide evidence for the presence of a population of mitochondria which exhibit coupled respiration *ex vivo* when isolated from the LV wall of advanced failing human hearts. Further, our results indicate that mitochondria from failing heart feature better complex I-mediated respiration than NFLV. What is unique about our work is that we were able to study respiratory function in freshly prepared LV mitochondria from hearts.

Our findings are in contradiction to much that has been published before. However most published studies have examined animal models of HF or non-LV tissue such as atrial appendage or septum tissue from humans with HF [9, 10, 28]. Essentially, the atrial appendage does not bear contractile load as the LV, therefore the energetics of these tissues are expected to be very different. Several elegant studies have examined mitochondrial function in the failing LV but have used surrogate markers such as enzyme activities, protein expression and complex activity assays to show reduced activities of some complexes in HF but not all [7, 8, 29–31]. Few studies before our current study have examined function of isolated mitochondria from human LV [11, 12, 14], of which, the study by Chidsey et al.

[14] showed that, *in vitro*, electron transport is tightly coupled to oxidative phosphorylation in mitochondria from left ventricular papillary muscles of patients with HF. Further, Lee et al. [11] demonstrated that LVAD support can enhance mitochondrial function, demonstrating that some degree of respiration control is maintained in human HF. In contrast, other studies using permeabilized fibers from HF patients have shown defects in mitochondrial oxidative phosphorylation and fatty acid metabolism [6, 9, 28]. In animal models, HF caused by drug-induced hypertension [32], aortic banding [33], coronary microembolization [3] or rapid ventricular pacing [34] have reported defective mitochondrial oxidative phosphorylation. However most protocols for animal models of HF are acute in nature, whereas most patients have chronic disease that develops over years and metabolic changes may have been initiated years before clinical diagnosis. Thus, the adaptation of the cardiac mitochondria in animal models to acute HF versus human adaptation to chronic HF may be different.

We find that increased mitochondrial respiration or RCR in HF mitochondria results primarily from reduced state 4 respiration rates (Fig. 2). Low state 4 rates, also reported in other studies [35], suggest reduced proton leak and tighter coupling, a function of an intact mitochondrial inner membrane [36]. Correspondingly, L/E ratios were significantly lower in HF mitochondria when PC or PM were used, indicative of tight coupling, lower proton leak, and less electron slippage. In contrast, state 4 rates for succinate were similar to those in NFLV suggesting differential adaptation of complex I vs. II respiration in HF. Our detailed functional analyses of HF mitochondria revealed increased RCR, P/E ratio and L/E ratio with reduced FCCP rates for GM. Glutamate enters the mitochondria via the glutamate-aspartate transporter, in which entry of glutamate- and H⁺ is coupled to the antiport of aspartate [37]. Influx of the accompanying H⁺ partly dissipates the proton gradient and therefore FCCP rates with GM maybe the lowest. In addition, FCCP-induced drop in membrane potential may lower the potential of the glutamate-aspartate transporter to exchange glutamate for aspartate, resulting in a greater limitation of fuel (glutamate) supply and lower FCCP rates for GM. Further, our data indicates a differential handling of PC vs. PM in HF mitochondria. FCCP rate for PM was significantly higher than PC even though state 3 and 4 rates were similar. It is likely that upstream impedance to entry of PC suppressed the maximal respiratory capacity, whereas such impedance may be absent for PM in HF. Further, P/E ratio for PM was lower than PC, which is suggestive of a loss of phosphorylation control of the HF mitochondria for PC relative to PM.

HF is not a specific disease but a complex syndrome with various co-morbidities and etiologies. Co-morbidities such as obesity and diabetes have deleterious effects on cardiac metabolism and mitochondrial function [10], and we found minor adaptive differences in mitochondrial respiration with etiology, obesity and diabetes, but not with duration of HF. In mitochondria from obese patients, RCR for PC were significantly greater, a potential adaptation to increased influx of lipids into cardiomyocytes in obesity. In addition, all patients received medications to control their HF symptoms although they still required LVAD support or transplant. Therefore our findings should be interpreted with the knowledge that all patients received medications (listed in Table 1).

Gene expression in HF LV showed down-regulation of both fatty acid and glucose metabolism pathways, consistent with previous studies reporting decreased expression of adult metabolic genes in HF [26]. Substrate switch from fatty acid to glucose utilization has been observed in acute HF studies; however, there is a paradoxical down-regulation of glucose oxidation genes despite increased flux in HF [38]. Suppressed fatty acid oxidation of PC may be responsible for the low FCCP rates for PC (Fig. 2D) compared to PM. It appears that *in situ*, the HF mitochondria are subjected to a milieu of metabolic impedances which are released once the mitochondria are isolated from the LV.

Our findings suggest that “energy-starvation” in HF is not due to the poor health of cardiac mitochondria themselves, since at least a sub-population of isolated mitochondria are capable of normal function when provided with ample substrates for oxidation. Further studies are required to assess whether the problem of energy-starvation in HF lies in substrate delivery to mitochondria, mitochondrial number or presence of factors that suppress mitochondrial function *in vivo* in the failing heart. It is possible that our isolation procedure selectively isolated a sub-population of mitochondria with normal function, and unhealthy mitochondria got eliminated in our procedure. Previous studies have shown reduction in mitochondrial number in HF [39, 40], and it may be possible that the remaining mitochondria in the failing heart have normal respiratory function but are insufficient to meet the high energy demands of the failing heart. Another untested explanation is that normal respiratory function in isolated HF mitochondria could result from allosteric or post-translational regulation of key mitochondrial enzymes as a consequence of brief exposure of mitochondria to Ca²⁺ during isolation or by activation of protein phosphatases. It is also possible that medications received by the NFLV and HF patients for improving heart function or for controlling co-morbidities may have an effect on the mitochondrial function assessed in our study. These issues will require further exploration, but do not detract from the finding that at least one population of mitochondria that we isolated from HF patients exhibit extraordinary retention of function *ex vivo*.

The mitochondrial isolation technique used in our study results in predominately SSMs, with limited IFMs. We chose not to study the subpopulations separately in this study since it allowed for mitochondrial isolates to be prepared for analysis in less than 50–60 minutes of surgeon hand-off. It also avoided any proteolytic effect should the enzyme for releasing IFMs enter the mitochondrial matrix. However, since we studied primarily SSMs, we cannot rule out the possibility that IFMs in HF may be dysfunctional while SSMs have preserved function. Some studies in animal models of heart failure have suggested greater impairment in IFM function than SSM function with HF[41] whereas other studies have suggested greater dysfunction in SSM after ischemia [42]. Future studies will examine whether SSM and IFM have differential function in the chronically failing humans hearts. We also chose to use isolated mitochondria rather than permeabilized fibers, because state 4 rates cannot be assessed with fibers [43], and studies with fibers are complicated by the presence of endogenous substrates which may interfere with respiration in response to exogenous substrates.

In conclusion, our observations offer a new perspective on energy substrate metabolism in the failing heart: mitochondria are not irreparably damaged in the failing heart and are

capable of coupled respiration when isolated and supplied with ample substrate *ex vivo*. However, there is no doubt that the failing heart is deprived of energy. Future studies will need to focus on regulators of substrate uptake and metabolism outside the respiratory chain that may impede ATP production *in vivo*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ACADI	Acyl-CoA dehydrogenase, long chain
ACADm	Acyl-CoA dehydrogenase, medium chain
ACADvl	Acyl-CoA dehydrogenase, very long chain
AMPK	5' AMP-activated protein kinase
CD36	Cluster of Differentiation 36
CPT2	Carnitine palmitoyltransferase II
ETC	Electron transport chain
FAD+	Flavin adenine dinucleotide
FCCP	Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone
GLUT1	Glucose transporter 1
GLUT4	Glucose transporter 4
GM	Glutamate-malate
HADHA	3-hydroxyacyl-coenzyme A dehydrogenase
HF	Heart failure
HFrEF	Heart failure with reduced ejection fraction
ICM	Ischemic cardiomyopathy
IFM	Interfibrillar mitochondria
L/E ratio	state 4 respiration to uncoupled rate
LV	Left Ventricle
LVIDd	LV internal diameter at diastole
ME3	Malic enzyme 3

NAD⁺	Nicotinamide adenine dinucleotide
NFLV	Non-failing Left Ventricle
NICM	Non-ischemic cardiomyopathy
P/E ratio	state 3 respiration to the uncoupled rate
PC	Palmitoylcarnitine-malate
PC	Pyruvate carboxylase
PDHB	Pyruvate Dehydrogenase subunit B
PDK4	Pyruvate dehydrogenase kinase 4
PGC1α	Peroxisome proliferator-activated receptor (PPAR)- γ coactivator 1 α
PHTN	Pulmonary hypertension
PM	Pyruvate-malate
PPARα	Peroxisome proliferator-activated receptor α
PPIA	Peptidylprolyl isomerase A (cyclophilin A)
RCR	Respiratory control ratio
RT-PCR	Reverse Transcriptase-Polymerized Chain Reaction
SSM	Subsarcolemmal mitochondria
Succ	succinate

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HIGHLIGHTS

- HF mitochondria have greater complex I respiration, higher ETC capacity with PM>PC
- Isolated mitochondria from HF have greater, coupled complex I respiration than NFLV
- Key genes of oxidation of fatty acids and glucose pathways are down-regulated in HF
- Obesity is associated with greater RCR for PC in HF
- Energy starvation in HF is not due to reduced mitochondrial ETC capacity

Figure 1. Mitochondrial function in NFLV tissue

Using palmitoylcarnitine-malate (PC), pyruvate-malate (PM), glutamate-malate (GM) and complex II substrate succinate (Succ), state 3 (A), state 4 (B) respiration rates were determined and RCR=state 3/state 4 (C) were calculated for NFLV mitochondria. Uncoupled rates were determined by addition of FCCP (D) and P/E ratio (E) and L/E ratio (F) were calculated.

N=4 samples per substrate and measurement. *P<0.05 vs. all other substrates.

Figure 2. Mitochondrial function in failing LV tissue

Mitochondria from failing LV tissue were assessed for state 3 (A), state 4 (B) respiration, RCR (C), FCCP rates (D), P/E ratio (E) and L/E ratio (F) for 4 substrates PC, PM, GM, Succ. N=20 samples per substrate and measurement. *P<0.05 vs. all other substrates, and † P<0.05 vs. PC for panel C-D, and vs. all other substrates for panels E-F.

Figure 3. Comparison of mitochondrial function in NFLV vs. HFLV

State 3 (A), state 4 (B), RCR (C), FCCP rates (D), P/E ratio (E) and L/E ratio (F) of HFLV mitochondria and NFLV mitochondria from Fig. 1 and 2 are shown together for comparison for all 4 substrates. Dotted line represents NFLV levels. N=20 HFLV, N=4 for NFLV samples per substrate and measurement. *P<0.05 vs. all other substrates.

Figure 4. Change in metabolic gene expression with HF

Expression of fatty acid uptake gene CD36 increased, but expression of fatty acid metabolism genes decreased in HF (A). Expression of glucose metabolism genes decreased in HF (B). Dotted line represents NFLV levels. N=20 HFLV, N=4 for NFLV samples. *P<0.05 vs. all other substrates.

Table 1

Demographic and clinical characteristics of the study population

Characteristic	NFLV* n=4	HF n=20	P-value
Age (yrs.)	45 ± 21	59 ± 11	0.06
Male gender (%)	0 (0)	18 (90)	0.0001
Race (%)			0.1
Caucasian	2 (50)	13 (65)	
African American	2 (50)	4 (20)	
Other	0 (0)	3 (15)	
Etiology			
Ischemic	0	12 (60)	
Non-ischemic	0	8 (40)	
Other	4	9 (45)	
BMI (kg/m ²)	27.1 ± 4.3	28.9 ± 7.1	0.6
Co-morbidities (%)			
Hypertension	1 (25)	14 (70)	0.07
Obesity	1 (25)	8 (40)	0.6
Dyslipidemia	0 (0)	10 (50)	0.07
T2DM	0 (0)	10 (50)	0.07
CKD	0 (0)	9 (45)	0.1
CVA	1 (25)	1 (5)	0.2
Atrial fibrillation	0 (0)	2 (10)	0.4
Hypothyroidism	0 (0)	2 (10)	0.5
Pulmonary †	2 (50)	5 (25)	0.4
Medications (%)			
ACEI	2 (50)	8 (40)	0.7
BB	3 (75)	17 (85)	0.6
Diuretics	2 (50)	17 (85)	0.2
Aldosterone inhibitors	0 (0)	8 (40)	0.2
Digoxin	2 (50)	11 (55)	0.8
Inotropes	1 (25)	3 (15)	0.6
Lipid lowering	0 (0)	7 (35)	0.2
Insulin	0 (0)	6 (30)	0.2
Metformin	0 (0)	1 (5)	0.6
Sulfonylureas	0 (0)	2 (10)	0.5
DDP-4 inhibitors	0 (0)	1 (5)	0.7
Echocardiographic parameters			
LVEF (%)	61 ± 12	19 ± 10	<0.0001
LVIDd (cm)	3.5 ± 1.2	6.6 ± 0.8	<0.0001

* Non-failing left ventricular (NFLV) samples were obtained from a cohort of patients with pulmonary hypertension, right ventricular failure and normal LV function. BMI: Body mass index, T2DM: type 2 diabetes mellitus, CKD: chronic kidney disease, CVA: cerebrovascular accident,

ACEI: angiotensin converting enzyme inhibitors, BB: beta-blockers, DDP-4: dipeptidyl peptidase 4 inhibitors, LVEF: left ventricular ejection fraction, LVIDd: left ventricular internal dimension.

[†] encompassed by COPD and secondary pulmonary hypertension.