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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 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.

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

Abbreviations: ACADl, 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 1α; 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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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 the 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 the 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 is 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 g 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 min.

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, the tissue was homogenized with a polytron homogenizer for two 5-second pulses, then centrifuged at 68 ×g at 4 °C for 10 min. The supernatant fraction was collected and centrifuged at 18,000 ×g for 10 min. 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 min. 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

Table 1
Demographic and clinical characteristics of the study population.

Characteristic	NFLV[a]	HF	P-value
	n = 4	n = 20	
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[b]	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

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.

[a] Non-failing left ventricular (NFLV) samples were obtained from a cohort of patients with pulmonary hypertension, right ventricular failure and normal LV function.

[b] Encompassed by COPD and secondary pulmonary hypertension.

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