

Quantifying the relationship and contribution of mitochondrial respiration to systemic exercise limitation in heart failure

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Abstract

Aims Heart failure with reduced ejection fraction (HFrEF) induces skeletal muscle mitochondrial abnormalities that contribute to exercise limitation; however, specific mitochondrial therapeutic targets remain poorly established. This study quantified the relationship and contribution of distinct mitochondrial respiratory states to prognostic whole-body measures of exercise limitation in HFrEF.

Methods and results Male patients with HFrEF ($n = 22$) were prospectively enrolled and underwent ramp-incremental cycle ergometry cardiopulmonary exercise testing to determine exercise variables including peak pulmonary oxygen uptake ($\dot{V}O_{2\text{peak}}$), lactate threshold ($\dot{V}O_{2\text{LT}}$), the ventilatory equivalent for carbon dioxide ($\dot{V}_E/\dot{V}CO_{2\text{LT}}$), peak circulatory power ($\text{CircP}_{\text{peak}}$), and peak oxygen pulse. *Pectoralis major* was biopsied for assessment of *in situ* mitochondrial respiration. All mitochondrial states including complexes I, II, and IV and electron transport system (ETS) capacity correlated with $\dot{V}O_{2\text{peak}}$ ($r = 0.40\text{--}0.64$; $P < 0.05$), $\dot{V}O_{2\text{LT}}$ ($r = 0.52\text{--}0.72$; $P < 0.05$), and $\text{CircP}_{\text{peak}}$ ($r = 0.42\text{--}0.60$; $P < 0.05$). Multiple regression analysis revealed that combining age, haemoglobin, and left ventricular ejection fraction with ETS capacity could explain 52% of the variability in $\dot{V}O_{2\text{peak}}$ and 80% of the variability in $\dot{V}O_{2\text{LT}}$, respectively, with ETS capacity ($P = 0.04$) and complex I ($P = 0.01$) the only significant contributors in the model.

Conclusions Mitochondrial respiratory states from skeletal muscle biopsies of patients with HFrEF were independently correlated to established non-invasive prognostic cycle ergometry cardiopulmonary exercise testing indices including $\dot{V}O_{2\text{peak}}$, $\dot{V}O_{2\text{LT}}$, and $\text{CircP}_{\text{peak}}$. When combined with baseline patient characteristics, over 50% of the variability in $\dot{V}O_{2\text{peak}}$ could be explained by the mitochondrial ETS capacity. These data provide optimized mitochondrial targets that may attenuate exercise limitations in HFrEF.

Keywords Exercise; HFrEF; Lactate threshold; Skeletal muscle; $\dot{V}O_{2\text{peak}}$

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Introduction

Heart failure with reduced ejection fraction (HFrEF) is a complex syndrome characterized by exercise intolerance and cardiac dysfunction. The severity of symptoms and exercise intolerance in HFrEF are most commonly assessed by the use of both subjective patient history (e.g. NYHA

classification) and more objective laboratory-based cardiopulmonary exercise testing (CPET). CPET is considered the gold-standard assessment of exercise intolerance in patients with HFrEF,^{1–3} providing an integrated multi-organ assessment. This facilitates refined non-invasive assessment of key indices of exercise limitation that independently predict prognosis, which include peak oxygen uptake ($\dot{V}O_{2\text{peak}}$), oxygen

uptake at the lactate threshold ($\dot{V}O_{2LT}$), and the ventilatory equivalent for carbon dioxide at the lactate threshold ($\dot{V}_E/\dot{V}CO_{2LT}$).^{4–11}

Although a defining feature of the HFrEF phenotype, the degree of left ventricular (LV) systolic dysfunction assessed by LV ejection fraction (LVEF) poorly correlates with the degree of exercise intolerance.^{12–14} In addition, pharmacological and device interventions that improve both LVEF and prognosis in HFrEF do not consistently improve exercise tolerance.^{15,16} As such, a multi-organ therapeutic approach in HFrEF has been advocated, which has identified peripheral skeletal muscle abnormalities as a key mechanism of exercise intolerance in HFrEF.^{17–21} Collectively, therefore, the need to clarify the relationship between skeletal muscle abnormalities and prognostic CPET measures in HFrEF could be useful for developing tailored therapeutic strategies aimed at improving exercise tolerance and quality of life.²²

Of particular interest is the major therapeutic target of skeletal muscle mitochondrial dysfunction,²³ which is proposed as a fundamental mechanism underlying persistent symptoms in HFrEF even when corresponding improvements in cardiac function are observed.²⁴ Recent technological advances provide a window for mitochondrial function to be dynamically assessed across the electron transport chain using muscle biopsies from patients with HFrEF, which allows important novelty to be gained compared with past studies where static morphological/biochemical measures were only able to confirm global reductions in mitochondrial content correlated to impairments in $\dot{V}O_{2peak}$.^{25–28} In contrast, the *in situ* interrogation of permeabilized myofibres allows evaluation of distinct respiratory states to identify key sites of limitation (i.e. across the electron transport chain at multiple mitochondrial complexes).^{29–31} In support, recent studies have confirmed that mitochondrial respiration from both the upper and lower limbs is reduced in patients with HFrEF, with impairments to complex I being closely correlated with $\dot{V}O_{2peak}$.^{30–32} Thus far, however, a comprehensive analysis of the relationship between mitochondrial respiratory states and key prognostic whole-body CPET variables remains poorly explored in HFrEF, and there is limited information about the relative contribution of specific mitochondrial respiratory deficits to the degree of exercise limitation.

The present study, therefore, directly assessed skeletal muscle mitochondrial respiration from biopsies of patients with HFrEF in order to evaluate the relationship and quantify the contribution of distinct respiratory complex states to whole-body prognostic CPET variables. We reasoned that a better understanding of the association between distinct mitochondrial deficits and whole-body exercise variables would optimize future targets for attenuating symptoms related to exercise intolerance in HFrEF.

Methods

Participants

We approached consecutive patients with stable signs and symptoms of chronic heart failure (>3 months receiving medical therapy) that were untrained but ambulatory, and presented with a LVEF <50% measured by two-dimensional echocardiography by Simpson's biplane following current guidelines³³ who were listed for a cardiac implantable electronic device at Leeds Teaching Hospitals NHS Trust, as described in full details elsewhere.³⁰ Participants were indicated for device therapy with either a pacemaker, implantable cardioverter defibrillator, or cardiac resynchronization therapy device according to current indications.³³ Exclusion criteria for participation in the present study included the inability to provide informed consent due to cognitive dysfunction or the presence of comorbidities potentially confounding assessment of exercise intolerance such as other cardiovascular conditions, chronic obstructive pulmonary disease, or ongoing malignancy or limiting musculoskeletal disease. All patients provided written informed consent, and all procedures were conducted in accordance with the Declaration of Helsinki after receiving local institute ethical approval (11/YH/0291). The patients included in this study are a subset of those on whom we have previously published other outcomes.^{30–32}

Cardiopulmonary exercise test

Cardiopulmonary exercise testing data for patients were obtained from a peak symptom-limited CPET conducted as part of routine clinical care, continued to volitional intolerance on a cycle ergometer for determination of $\dot{V}O_{2peak}$, $\dot{V}O_{2LT}$, peak circulatory power ($CircP_{peak}$), and peak oxygen (O_2) pulse, RER_{peak} , and $\dot{V}_E/\dot{V}CO_{2LT}$. After a 5-min warm-up at 10 W, the patients started cycling at 20 W and workload was progressively increased by 5 W min^{-1} until the patients reached volitional intolerance. Ventilatory and pulmonary gas exchange variables were collected as 15 s averages on a breath-by-breath basis using a calibrated system (Ultimo CardO₂, Medical Graphics, St. Paul, MN, USA). $\dot{V}O_{2peak}$ and RER_{peak} were recorded as the highest 15 s average, while $\dot{V}O_{2LT}$ was calculated using the V-slope method, which also allowed $\dot{V}_E/\dot{V}CO_{2LT}$ to be determined.³⁴ In the instance when a patient did not achieve lactate threshold and/or we were unable to estimate lactate threshold ($n = 10$), the $\dot{V}O_{2LT}$ and $\dot{V}_E/\dot{V}CO_{2LT}$ were not included in the analysis. $CircP_{peak}$ was defined as the product of $\dot{V}O_{2peak}$ and peak systolic blood pressure^{35–38} whereas peak O_2 pulse was calculated as $\dot{V}O_{2peak}$ divided by heart rate peak. Patients removed the

mouthpiece at the end of the test during recovery due to discomfort; $\dot{V}O_2$ and $\dot{V}CO_2$ recovery half time are therefore not reported.

Muscle biopsy

Skeletal muscle biopsy of the *pectoralis major* (~50 mg) was obtained during the routine device implantation procedures as previously described,³⁰ which took place within one month of baseline clinical data collection. There were no complications or adverse events with this procedure. A portion of muscle tissue was immediately placed in 1 mL of ice-cold specialized preservation solution (BIOPS) for subsequent assessment of mitochondrial respiration.³¹ Recent evidence from patients with HFrEF indicate sampling of the upper limb muscles provide an opportunity to investigate the disease's systemic myopathy, with the *pectoralis major* providing a close surrogate for the vastus lateralis in terms of mitochondrial function and closely linked to whole-body exercise intolerance that is not as impacted by other confounding factors (e.g. disuse, detraining, or arthritis).³⁰ Furthermore, muscle mass of the *pectoralis major* is also a powerful predictor of prognosis in HFrEF³⁹ and in relation to daily activities, the upper limbs typically perform various tasks (e.g. house chores and gardening) that would pose exercise limitations impacting patient quality of life. As such, the clinical relevance for sampling the *pectoralis major* in terms of exercise limitations and prognosis is well supported.

Mitochondrial function

Mitochondrial respiration was measured *in situ* from saponin-permeabilized skeletal muscle fibres using high-resolution respirometry (Oxygraph-2K; Oroboros Instruments, Innsbruck, Austria) as previously described elsewhere.^{30–32} Briefly, the following steps were performed including (i) Complex I leak respiration was determined by addition of glutamate (10 mM), malate (0.5 mM), and pyruvate (5 mM) (i.e. a measure of proton leak under non-phosphorylating conditions); (ii) adenosine diphosphate (2.5 mM) was added to stimulate oxidative phosphorylation through Complex I (OXPHOS P_I); (iii) outer mitochondrial membrane integrity was determined by addition of 10 μ M cytochrome c; (iv) succinate at 10 mM to stimulate Complex I + II (OXPHOS P_I + II); (v) 0.5 μ M titrations of FCCP to achieve maximal uncoupled respiration for electron transport system (ETS) capacity; (vi) Complex I inhibitor rotenone at 0.25 μ M for ETS-supported Complex II respiration (C_{II}); (vii) 2.5 μ M antimycin A as a Complex III inhibitor for residual oxygen consumption (ROX) to calculate non-mitochondrial (background) respiration, which was subtracted from all values. Complex IV respiration (C_{IV}) was

also measured by the addition of 0.5 mM TMPD and 2 mM ascorbate.⁴⁰ Values for mitochondrial respiration were normalized to muscle wet mass and are presented as pmol O₂/s/mg.

Statistical analysis

Bivariate associations between mitochondrial and CPET variables were examined by Pearson's correlations coefficients. Multiple regression analysis was carried out to determine the independent contributors of $\dot{V}O_{2peak}$ variability. In a first step, variables with an established physiological role in $\dot{V}O_{2peak}$ including age, haemoglobin concentration (Hb), and LVEF were entered as a block to a multivariable regression model to determine how much of the variability in $\dot{V}O_{2peak}$ could be explained by these variables.^{41–43} We then ran the same model combined with each mitochondrial complex separately to quantify the contribution of each complex by explaining $\dot{V}O_{2peak}$ variability. If any of the r^2 value was greater than 0.75 and thus the variance inflation factor greater than 4.0, we considered multicollinearity might be a problem, but this was not the case in the present analysis. Statistical significance was accepted as $P < 0.05$. Data are presented as mean \pm standard deviation. All statistical analyses were performed using GraphPad Prism version 9.0.0.

Results

Patient population

Patient characteristics and medications are shown in *Table 1*, while CPET data are shown in *Table 2*.

Correlation between mitochondrial respiration and cardiopulmonary exercise testing variables

We first assessed the correlation between key patient physical and clinical variables with CPET measures. There were no correlations ($P > 0.05$) between age, Hb, and LVEF with $\dot{V}O_{2peak}$ or other CPET variables, although Hb showed a strong trend with $\dot{V}O_{2peak}$ ($r = 0.38$; $P = 0.08$). In contrast, peripheral skeletal muscle mitochondrial function of the *pectoralis major* was well correlated with various whole-body CPET variables (*Table 3*). *Figure 1* displays the correlations between stimulated mitochondrial respiratory states and the most common measure of exercise intolerance: $\dot{V}O_{2peak}$. Mitochondrial respiration of complex I, complex I + II and ETS capacity correlated with $\dot{V}O_{2peak}$ (range: $r = 0.49–0.64$ and $P < 0.01$; *Table 3* and *Figure 1A–C*), whereas complexes II and IV did not correlate (*Table 3*; *Figure 1D–E*).

Table 1 Clinical variables of patients with heart failure with reduced ejection fraction (n = 22)

Demographics	
Age (years)	69 ± 12
Male sex [n(%)]	22 (100)
BMI (kg/m ²)	28.5 ± 4.0
NYHA Class III/IV [n(%)]	5 (23)
Aetiology of HF	
Ischaemic [n(%)]	9 (41)
DCM [n(%)]	6 (27)
Past medical history	
Diabetes mellitus [n(%)]	6 (27)
AF [n(%)]	11 (50)
COPD [n(%)]	1 (5)
Hypertension [n(%)]	10 (45)
Device therapy	
PPM [n(%)]	3 (14)
ICD [n(%)]	4 (18)
CRT [n(%)]	15 (68)
Medications	
Antiplatelet [n(%)]	11 (50)
Beta-blocker [n(%)]	18 (82)
ACE-I/ARB [n(%)]	18 (82)
Loop diuretic [n(%)]	11 (50)
MRA [n(%)]	11 (50)
Statin [n(%)]	16 (73)
Anticoagulant [n(%)]	8 (36)
Metformin [n(%)]	4 (18)
Insulin [n(%)]	2 (9)
Echocardiogram	
LVEF (%)	31.7 ± 13.9
LVEDd (mm)	58.1 ± 7.1
PASP (mmHg)	33.6 ± 15.9
Laboratory investigations	
Hb (g/L)	140.8 ± 15.4
Creatinine (μmol/L)	98.5 ± 24.1
HbA1c (mmol/mol)	45.1 ± 11.1

Normally distributed continuous variables are expressed as mean ± standard deviation; discrete variables are presented as number and percentages in parentheses. ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; CHF, chronic heart failure; COPD, chronic obstructive pulmonary disease; CRT, cardiac resynchronisation therapy; DCM, dilated cardiomyopathy; Hb, haemoglobin; HbA1c, glycated haemoglobin; ICD, implantable cardioverter defibrillator; LVED, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor blocker; NT-pro-BNP, N-terminal pro brain natriuretic peptide; NYHA, New York Heart Association; PASP, pulmonary artery systolic pressure; PPM, permanent pacemaker.

We next assessed the correlation between mitochondrial respiration and another key CPET-derived measure of aerobic performance and prognosis in HFrEF: the $\dot{V}O_{2LT}$ (Figure 2). Mitochondrial respiratory complexes I, I + II, and IV and ETS capacity were all correlated with $\dot{V}O_{2LT}$ (range: $r = 0.60$ – 0.71 and $P < 0.05$; Table 3 and Figure 2A–E), whereas complex II did not ($P = 0.084$; Table 3 and Figure 2D). In addition to $\dot{V}O_{2peak}$ and $\dot{V}O_{2LT}$, the $\dot{V}_E/\dot{V}CO_{2LT}$ is another strong predictor of prognosis in HFrEF that is thought to be independent of effort. As opposed to $\dot{V}O_{2peak}$ and $\dot{V}O_{2LT}$, we did not observe correlations across mitochondrial respiratory complexes with $\dot{V}_E/\dot{V}CO_{2LT}$ ($P > 0.05$; Table 3) or with another variable that is commonly used to indicate maximal effort: RER_{peak} ($P > 0.05$;

Table 2 Exercise variables for patients with heart failure with reduced ejection fraction

CPET variable	Mean ± SD
$\dot{V}O_{2peak}$ (mL/kg/min)	16.7 ± 4.9
$\dot{V}O_{2LT}$ (mL/kg/min)	12.6 ± 4.9
$CircP_{peak}$ (mmHg × mL/kg/min)	2435 ± 863
Peak O ₂ Pulse (mL/beat)	11.9 ± 5.5
RER_{peak}	1.1 ± 0.2
$\dot{V}_E/\dot{V}CO_{2LT}$	32.2 ± 6.1
SBP_{peak} (mmHg)	143.1 ± 22.4
DBP_{peak} (mmHg)	72.0 ± 8.8
HR_{peak} (bpm)	120.1 ± 21.6

CPET, cardiopulmonary exercise testing; peak O₂ pulse, peak oxygen pulse; $CircP_{peak}$, peak circulatory power; RER_{peak} , respiratory exchange ratio at $\dot{V}O_{2peak}$; $\dot{V}_E/\dot{V}CO_{2LT}$, ventilation and carbon dioxide production ratio at lactate threshold; $\dot{V}O_{2peak}$, peak oxygen uptake; $\dot{V}O_{2LT}$, lactate threshold.

Table 3 Correlations between *pectoralis major* mitochondrial respiratory states and cardiopulmonary exercise testing variables, including r , r^2 and level of significance

	P _I	P _{I + II}	ETS	C _{II}	C _{IV}
$\dot{V}O_{2peak}$					
r	0.58	0.54	0.64	0.49	0.40
r^2	0.34	0.29	0.41	0.24	0.16
P	0.00	0.01	0.00	0.02	0.06
$\dot{V}O_{2LT}$					
r	0.72	0.71	0.70	0.52	0.60
r^2	0.52	0.49	0.49	0.27	0.36
P	0.01	0.01	0.01	0.08	0.04
$CircP_{peak}$					
r	0.60	0.56	0.54	0.42	0.49
r^2	0.36	0.31	0.29	0.18	0.25
P	0.01	0.02	0.02	0.08	0.04
Peak O ₂ pulse					
r	0.25	0.11	0.31	0.17	0.29
r^2	0.06	0.01	0.09	0.28	0.08
P	0.36	0.69	0.25	0.53	0.28
RER_{peak}					
r	−0.09	−0.25	−0.12	−0.06	−0.18
r^2	0.00	0.06	0.01	0.00	0.03
P	0.76	0.38	0.68	0.84	0.54
$\dot{V}_E/\dot{V}CO_{2LT}$					
r	−0.15	−0.21	−0.24	−0.48	0.21
r^2	0.02	0.04	0.06	0.23	0.04
P	0.64	0.51	0.45	0.12	0.52

$CircP_{peak}$, peak circulatory power; C_{II}, ETS-supported Complex II; C_{IV}, Complex IV; ETS, electron transport system capacity; RER_{peak} , respiratory exchange ratio at $\dot{V}O_{2peak}$; $\dot{V}_E/\dot{V}CO_{2LT}$, ventilation and carbon dioxide production ratio at lactate threshold; $\dot{V}O_{2peak}$, peak oxygen uptake; $\dot{V}O_{2LT}$, lactate threshold; peak O₂ pulse, peak oxygen pulse; P_I, OXPHOS Complex I; P_{I + II}, OXPHOS complex; r , r correlation; r^2 , r squared; P , P value.

Table 3). While the peak O₂ pulse also showed no correlations ($P > 0.05$; Table 3), we found $CircP_{peak}$ was correlated across most mitochondrial respiratory complexes (range: $r = 0.42$ – 0.60 and $P < 0.05$; Table 3). An overview of the degree of correlation between invasive skeletal muscle mitochondrial measures and non-invasive whole-body performance CPET variables is presented in Figure 3, with

Figure 1 Correlations between mitochondrial respiratory states and whole-body $\dot{V}O_{2peak}$. P_{I_1} , OXPHOS complex I; $P_{I_1 + II}$, OXPHOS complex; ETS, maximal electron transport system capacity; C_{II} , ETS-supported complex II; C_{IV} , complex IV.

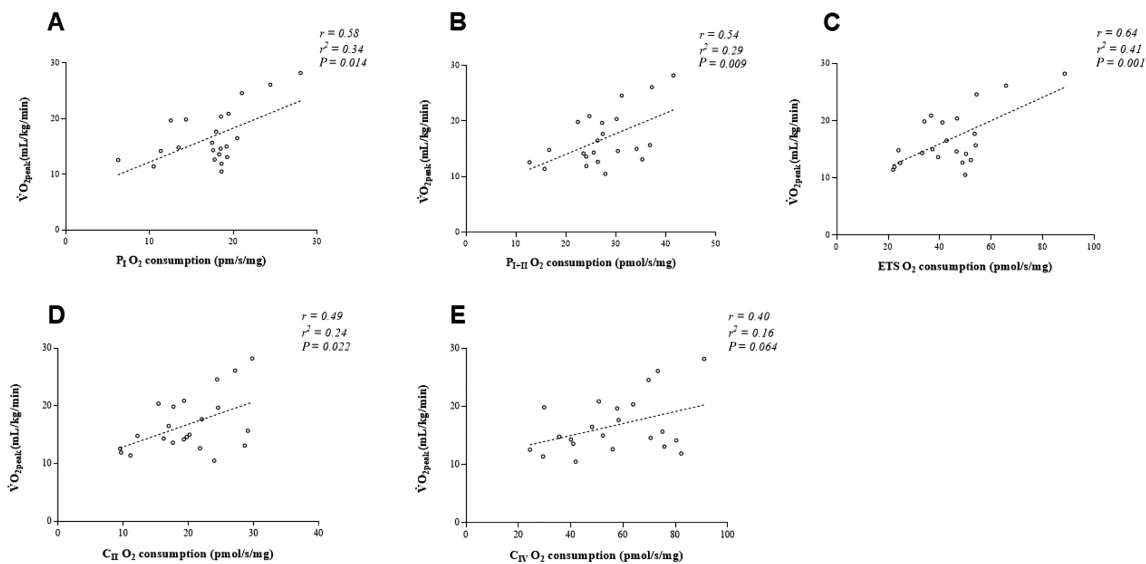
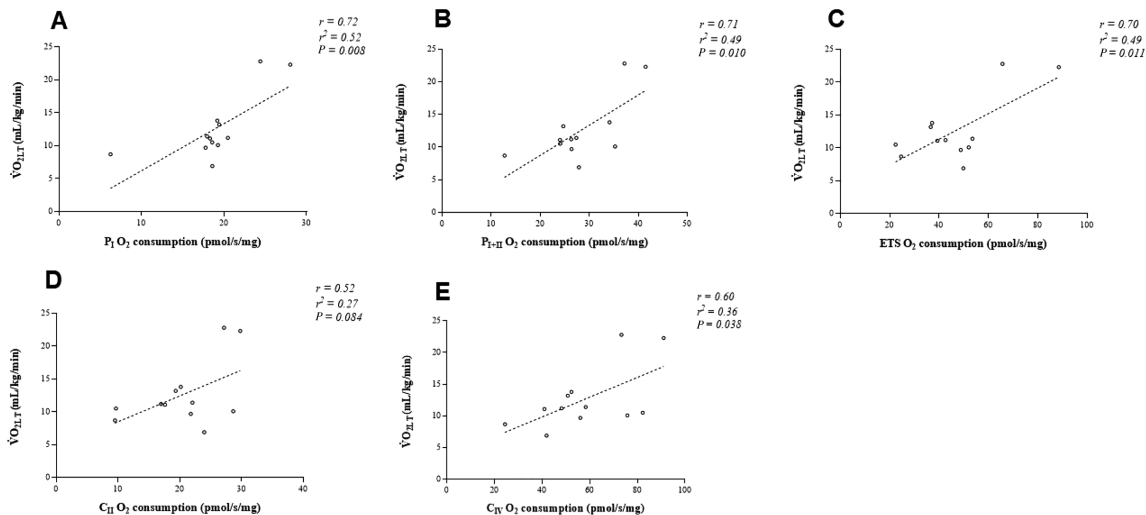


Figure 2 Correlations between mitochondrial respiratory states and lactate threshold ($\dot{V}O_{2LT}$). C_{II} , ETS-supported Complex II; C_{IV} , Complex IV; ETS, maximal electron transport system capacity; P_{I_1} , OXPHOS Complex I; $P_{I_1 + II}$, OXPHOS complex.



the strongest relationships found between the mitochondrial respiratory states of ETS capacity and complex I with $\dot{V}O_{2peak}$ and $\dot{V}O_{2LT}$, respectively.

Multiple regression analysis

A multiple regression was performed with the baseline patient variables including age, Hb, LVEF as one block and alongside each mitochondrial respiratory state in the

pectoralis major to predict the variability in determining whole-body $\dot{V}O_{2peak}$ and $\dot{V}O_{2LT}$. Patient characteristics of age, Hb, and LVEF could explain 24% of the variability in $\dot{V}O_{2peak}$; however, the model or one of the contributors did not reach statistical significance. *Figure 4A* demonstrates the r^2 value and level of significance for the model combined with each individual mitochondrial complex. Overall, the model that included age, Hb, LVEF, and ETS capacity was able to explain 52% of the variability in $\dot{V}O_{2peak}$ (r^2 0.52; $P = 0.01$). From all mitochondrial variables, ETS capacity and complex I

Figure 3 Heat map showing intensity of correlation between mitochondrial respiratory states and whole-body cardiopulmonary exercise testing (CPET) variables.

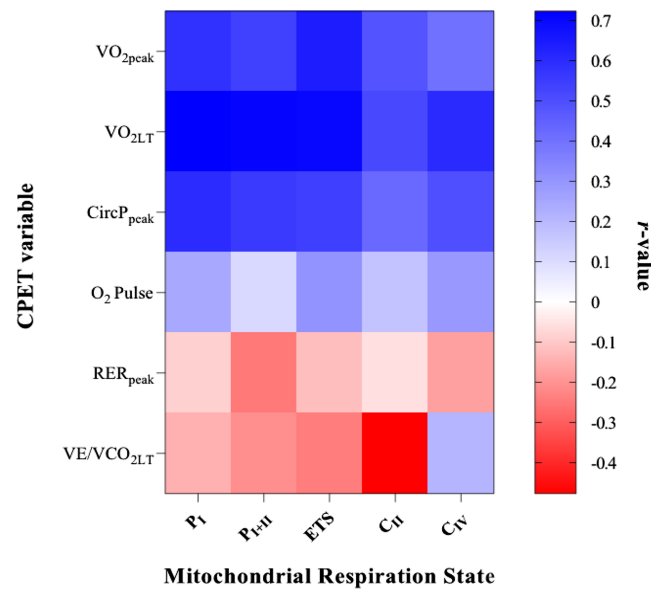
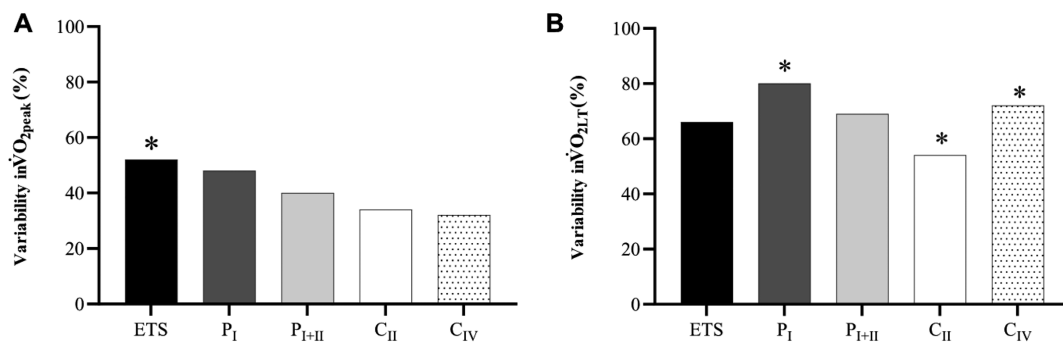


Figure 4 Bar chart demonstrating the percentage $\dot{V}O_{2peak}$ (A) and $\dot{V}O_{2LT}$ (B) explained by individual mitochondrial complexes combined with age, Hb and LVEF. * $P < 0.05$ for analysis of variance, such that independent variables significantly predict $\dot{V}O_{2peak}$.



were the only independent variables that reached statistical significance while all others resulted in a lower prediction of $\dot{V}O_{2peak}$ (range: 30–50% and $P > 0.05$; Figure 4A). In addition, mitochondrial complex I respiration explained 80% of the variability in $\dot{V}O_{2LT}$ (r^2 0.80 and $P = 0.01$; Figure 4B) and complex IV 72% (r^2 0.72 and $P = 0.04$; Figure 4B), while the other individual mitochondrial complexes did not reach statistical significance.

Discussion

We confirm that invasive measurements of mitochondrial respiration from skeletal muscle biopsies of patients with

HFrEF independently correlate with standard non-invasive clinical CPET variables that are commonly used to assess exercise limitation and prognosis. We show that measures of mitochondrial respiration under a range of stimulated conditions (i.e. complexes I, II, and IV and ETS capacity) were correlated with $\dot{V}O_{2peak}$, $\dot{V}O_{2LT}$, and CircP_{peak}. In contrast, other important prognostic CPET-derived indices such as peak O₂ pulse, $\dot{V}_E/\dot{V}CO_{2LT}$ and indicators of maximal effort (i.e. RER_{peak}) showed no clear relationship. Importantly, when combined with baseline patient characteristics, mitochondrial ETS capacity could explain at least 50% of the variability in $\dot{V}O_{2peak}$ (i.e. one of the strongest measures of exercise limitation and prognosis in HFrEF). Overall, therefore, our findings suggest that therapies targeting specific limitations in mitochondrial respiration related to ETS capacity could benefit

whole-body exercise tolerance and prognosis in patients with HFrEF.

Is mitochondrial function linked to cardiopulmonary exercise testing variables in heart failure with reduced ejection fraction?

Exercise tolerance depends on the succession of several steps in the oxygen delivery-utilization cascade from lungs, heart, and vasculature to skeletal muscle mitochondria,⁴⁴ which is most commonly quantified using CPET⁴² where key exercise variables that include $\dot{V}O_{2peak}$, $\dot{V}O_{2LT}$, and $\dot{V}_E/\dot{V}CO_{2LT}$ strongly predict mortality.⁴ Mitochondrial abnormalities in peripheral skeletal muscle are a major mechanism limiting whole-body $\dot{V}O_{2peak}$ in patients with HFrEF.^{17–21} Surprisingly few studies have provided a comprehensive assessment of the relationship between mitochondrial function and prognostic CPET variables to delineate mitochondrial-specific impairments vs. CPET indices beyond $\dot{V}O_{2peak}$. We hypothesized that careful examination of the relationships between invasive mitochondrial and whole-body CPET variables would provide important insight for understanding markers of exercise limitation and prognosis in HFrEF.

It has previously been shown that static measures of mitochondrial content and morphology are correlated to $\dot{V}O_{2peak}$ in patients with HFrEF.^{25–28} Here we uniquely developed this line of enquiry by examining the contribution of dynamic mitochondrial respiration across complexes I, II, and IV and ETS in skeletal muscle to prognostic measures of exercise limitation in patients with HFrEF. Recent detailed assessments of mitochondrial respiration *in situ* have confirmed oxygen flux is lower in patients with HFrEF.^{30–32} Impairments to mitochondrial content rather than intrinsic function have been suggested to play the dominant role, with complex I and IV dysfunction being closely correlated to $\dot{V}O_{2peak}$.^{30–32} Earlier studies using more static measurements of mitochondrial function (e.g. *in vitro* enzyme activity or protein content) have also found lower values in patients with HFrEF that correlated to $\dot{V}O_{2peak}$,^{25,26} but findings have been inconsistent in both humans⁴⁵ and animals.^{46,47} However, beyond the $\dot{V}O_{2peak}$, relatively little information has been gleaned from other CPET measures and their relationship to mitochondrial function in HFrEF. This has limited insights into whether mitochondrial abnormalities in HFrEF could also be contributing to other prognostic CPET markers. Here we clearly show that multiple mitochondrial respiration states (e.g. complexes I, II, and IV and ETS) were correlated to the key CPET parameters of $\dot{V}O_{2LT}$ and $\dot{V}O_{2peak}$, but not to the ventilatory prognostic marker of $\dot{V}_E/\dot{V}CO_{2LT}$. The reason for this is likely explained by both $\dot{V}O_{2LT}$ and $\dot{V}O_{2peak}$ being dictated, in large part, by the degree of oxygen utilization (and thus mitochondrial function), while $\dot{V}_E/\dot{V}CO_{2LT}$ being more closely linked to pulmonary/acid–base balance coupling.⁴⁸ What is clear is that

interventions to improve $\dot{V}O_{2peak}$ in patients with HFrEF are frequently associated with improvements in prognosis: for example, exercise training in patients with HFrEF reverses skeletal mitochondrial abnormalities in parallel to improved $\dot{V}O_{2peak}$ and prognosis.^{49–51}

One intriguing question, therefore, is whether improving skeletal muscle mitochondrial respiration alone would increase $\dot{V}O_{2LT}$ and $\dot{V}O_{2peak}$ in patients with HFrEF, thereby improving symptoms and prognosis? Rodent models that inherit a genetically high $\dot{V}O_{2peak}$ are known to have higher levels of mitochondrial function in skeletal muscle, and these are linked to reduced development of cardiometabolic disease and improved survival rates.⁵² Thus, these data support the concept that maintaining high ‘mitochondrial fitness’ in skeletal muscle would likely benefit both measures of exercise tolerance and prognosis in patients with HFrEF.

The contribution of mitochondrial function to exercise limitation in heart failure with reduced ejection fraction

Improving our understanding of the primary factors limiting exercise tolerance in HFrEF will facilitate the identification of more targeted mechanisms and the advent of more effective treatments. Our study and work by others clearly highlight a close relationship between skeletal muscle mitochondrial dysfunction and whole-body exercise tolerance in HFrEF.^{17–21} However, this study provides further information by using experimental evidence to predict the distinct intrinsic mitochondrial characteristics and their contribution towards the degree of exercise intolerance developed in HFrEF. Here, our findings of the monovariate analysis add new evidence by demonstrating that mitochondrial function under various respiratory states (e.g. P_I , $P_I + II$, and ETS) contributes around 50% and 35% to the variability in $\dot{V}O_{2LT}$ and $\dot{V}O_{2peak}$ in HFrEF, respectively. Given that $\dot{V}O_{2peak}$ and $\dot{V}O_{2LT}$ are influenced by other key factors such as cardiac output, blood flow distribution, and oxygen diffusion,⁴⁴ the fact that skeletal muscle mitochondrial function does not entirely explain all the variance in whole-body $\dot{V}O_{2peak}$ and $\dot{V}O_{2LT}$ is not surprising.

To provide further evidence we also performed a multivariate regression analysis that included key patient characteristics (e.g. age, Hb, and LVEF), which demonstrated that 52% of the variability in $\dot{V}O_{2peak}$ in HFrEF was related to mitochondrial ETS capacity (vs. 41% at the monovariate level). Interestingly only mitochondrial ETS capacity was independently correlated to $\dot{V}O_{2peak}$ in the multivariate analysis and not other respiratory states. These data indicate, therefore, that mitochondrial ETS capacity should be considered one major target for future skeletal muscle mitochondrial therapies in HFrEF, as this would likely achieve the greatest benefits to whole-body exercise tolerance. The unexplained variance in

the model is likely explained by other factors, including inter-individual pathophysiological differences related to the O₂ delivery-utilization cascade related to skeletal muscle, cardiac and pulmonary mechanisms.

Recent data from a larger cohort of patients with HFrEF have also shown that training status and smoking history can further play a role in explaining the $\dot{V}O_{2\text{peak}}$ variability.⁵³ Indeed, impaired peripheral vascular function is also touted not only to play a major role in causing severe exercise limitations in patients with HFrEF, but as a potentially important upstream mechanism that could mediate severe mitochondrial derangements (i.e. by reducing oxygen delivery via endothelial dysfunction and/or capillary rarefaction).⁴⁴ As such, we acknowledge that mitochondrial respiration cannot entirely explain $\dot{V}O_{2\text{peak}}$ variability in HFrEF patients and more research is needed to elucidate other contributing mechanisms using larger sample sizes. Yet, our findings could contribute to better understanding what specific mechanisms allow patients with HFrEF to increase their $\dot{V}O_{2\text{peak}}$ following exercise training given the wide variability typically observed, as recently reviewed in detail.⁵⁴

Conclusion

Invasive measurements of mitochondrial respiratory states from skeletal muscle biopsies of patients with HFrEF were independently correlated to key non-invasive CPET measures of

exercise limitation and prognosis that included $\dot{V}O_{2\text{peak}}$, $\dot{V}O_{2\text{LT}}$, and $\text{CircP}_{\text{peak}}$, but not $\dot{V}_E/\dot{V}CO_{2\text{LT}}$, peak O₂ pulse or RER_{peak} . When combined with other patient variables, at least 50% of the variability in $\dot{V}O_{2\text{peak}}$ could be explained by the mitochondrial ETS capacity. Overall, these data expand our knowledge on specific mitochondrial targets in the skeletal muscle that may help alleviate symptoms of exercise intolerance in HFrEF.

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Conflict of interest

P. K. declares no conflict of interest. S. S. declares no conflict of interest. J. G. declares no conflict of interest. A. K. declares no conflict of interest. L. D. R. declares no conflict of interest. K. K. W. has received speakers' fees and honoraria from Medtronic, Cardiac Dimensions, Novartis, Abbott, BMS, Pfizer, Bayer, and has received unconditional research grants from Medtronic. C. F. declares no conflict of interest. T. S. B. declares no conflict of interest.

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