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REDUCED EXERCISE TOLERANCE IN CHF MAY BE RELATED TO FACTORS OTHER THAN IMPAIRED SKELETAL MUSCLE OXIDATIVE CAPACITY

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Brief title

Exercise Limitation in Chronic Heart Failure

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ABSTRACT

Background. We sought to determine whether skeletal muscle oxidative capacity, fiber type proportions and fiber size, capillary density or muscle mass might explain the impaired exercise tolerance in chronic heart failure (CHF). Previous studies are equivocal regarding the maladaptations that occur in the skeletal muscle of patients with CHF and their role in the observed exercise intolerance.

Methods and Results. Total body O₂ uptake ($\dot{V}O_2$ peak) was determined in 14 CHF patients and 8 healthy sedentary similarly-aged controls. Muscle samples were analysed for mitochondrial ATP production rate (MAPR), oxidative and glycolytic enzyme activity, fiber size and type, and capillary density. CHF patients demonstrated a lower $\dot{V}O_2$ peak (15.1 ± 1.1 vs. 28.1 ± 2.3 ml.kg⁻¹.min⁻¹, p<0.001) and capillary to fiber ratio (1.09 ± 0.05 vs. 1.40 ± 0.04 ; p<0.001) when compared to controls. However there was no difference in capillary density (capillaries per μm^2) across any of the fiber types. Measurements of MAPR and oxidative enzyme activity indicated no difference in muscle oxidative capacity between the groups. **Conclusions.** Neither reduced muscle oxidative capacity nor capillary density are the cause of exercise limitation in CHF patients. We conclude that the low $\dot{V}O_2$ peak observed in CHF patients is the result of muscle fiber atrophy and possibly impaired activation of oxidative phosphorylation.

KEY WORDS: Exercise Capacity, Skeletal Muscle

INTRODUCTION

Compared to healthy subjects, patients with chronic heart failure (CHF) exhibit a reduced exercise tolerance characterised by a low peak oxygen consumption ($\dot{V}O_{2\text{ peak}}$) and the early onset of fatigue¹ and lactic acidosis² during incremental exercise. These symptoms were formerly considered to be due to impaired cardiac function in these patients. There is no correlation, however, between $\dot{V}O_{2\text{ peak}}$ and indices of left ventricular performance³. Further, in CHF patients the addition of arm exercise to maximal leg exercise produces increases in $\dot{V}O_{2\text{ peak}}$ suggesting that exercise capacity in these subjects is limited by muscle function not cardiac output⁴.

We have previously observed deficits in exercise tolerance in lung transplant recipients⁵ similar to those described above for CHF patients. The vastus lateralis muscles of the lung transplant recipients exhibited a range of abnormalities which potentially contributed to their reduced exercise capacity. These abnormalities included reduced levels of oxidative enzymes, a reduced proportion of type I muscle fibers and, significantly, lowered mitochondrial ATP production rates (MAPR). The exercise intolerance in CHF has also been attributed to maladaptations in skeletal muscle such as reduced oxidative capacity, altered muscle fiber type composition and fiber atrophy or loss leading to reduced muscle strength⁶⁻⁹. It is also possible that reduced muscle blood flow is also an important component in exercise impairment in these patients⁸.

The effects of CHF on skeletal muscle oxidative capacity are unclear. Whilst most reports have suggested reduced activity of oxidative enzymes, this is not universally the case.

Sullivan et al.¹⁰ report reductions in both CS and SDH activity in CHF patients while Opasich and colleagues²⁹ reported no difference in either of these enzymes in their CHF group. Other similar studies have reported significant reductions in some but not all oxidative enzymes measured^{11,13,14}. Interestingly, studies examining the effects of CHF on oxidative phosphorylation *in vivo* have consistently reported reduced rates of oxidative phosphorylation in CHF patients^{27,28}. The anomalies alluded to above notwithstanding; reduced exercise tolerance has therefore been attributed to reduced muscle oxidative capacity. The rate of ATP production is a very sensitive test of mitochondrial function in skeletal myocytes¹⁹. However, no previous studies have examined mitochondrial ATP production rates (MAPR) in CHF patients.

The aim of the present study was to determine the role of skeletal muscle histochemical and metabolic maladaptations to CHF. In particular, we hypothesized that, compared to sedentary similarly aged healthy controls, the skeletal muscle of CHF patients would exhibit lower MAPR, and that this would be associated with a higher proportion of glycolytic fibers, muscle fiber atrophy and lower capillary density.

MATERIALS AND METHODS

Subjects. Fourteen stable chronic heart failure (CHF) patients (13 male/1 female; 68 ± 9 years, 84 ± 15 kg, body mass index 28 ± 5 kg/m²; mean \pm S.D.) and eight healthy sedentary control subjects (7 male/1 female; 63 ± 11 years, 78 ± 8 kg, body mass index 26 ± 3 kg/m²) participated in this study. The CHF group were in New York Heart Association functional class (NYHA) II (n = 11) & III (n = 3) and had left ventricular ejection fractions (LVEF) of $27 \pm 8\%$. The average time since diagnosis of CHF in the patients was 39 ± 41 months. Two of the 14 CHF patients were current smokers while all healthy subjects were non-smokers. Diagnoses and medications for the CHF patients are presented in Table 1. Control subjects were healthy sedentary individuals with no history of left ventricular dysfunction. Written informed consent was obtained from all subjects prior to their entry into this study which was approved by the Human Research Ethics Committees of The Austin and Repatriation Medical Centre and Victoria University of Technology. The investigation conforms to the principles outlined in the Declaration of Helsinki.

Incremental Exercise Tests and blood sampling. Peak total body oxygen consumption ($\dot{V}O_{2\text{ peak}}$) was determined on CHF patients during a symptom-limited graded exercise test on an electronically braked bicycle ergometer (Ergomed, Siemens, Erlangen, Germany), commencing at 10 W and increasing by $10 \text{ W}\cdot\text{min}^{-1}$ to volitional fatigue or until symptoms intervened. In the healthy control group, the exercise test commenced at 20 W and increased by $20 \text{ W}\cdot\text{min}^{-1}$ until a respiratory exchange ratio (RER) of 1.0 was reached. Work rate then increased by $10 \text{ W}\cdot\text{min}^{-1}$ until volitional fatigue or a level of 17

on the 6-20 point Borg scale of perceived exertion ¹⁵. Expired volume and expired oxygen and carbon dioxide concentrations were analysed and used to compute oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and respiratory exchange ratio (RER = $\dot{V}CO_2/\dot{V}O_2$) as previously described ¹⁶. Arterial oxygen saturation (pulse oximetry; Oxi-Radiometer, Boulder, CO) and ECG were monitored continuously throughout the test.

Blood samples were obtained from a dorsal hand vein via a 20-gauge indwelling catheter. Arterialisation was maintained by immersion of the hand, covered by a waterproof plastic glove, in a water bath maintained at 45°C. Blood samples were drawn at rest, every minute during exercise and at 1, 2, 5 and 10 minutes post-exercise for measurement of arterial oxygen saturation (SaO₂) and plasma lactate concentration. Oxygen saturation in the blood samples was consistently in excess of 95%, confirming arterialisation.

Plasma Lactate, Calculation of Lactate Threshold and Predicted $\dot{V}O_{2\text{ peak}}$. Plasma lactate levels were determined using Roche Diagnostic Kit reagents. Lactate threshold was calculated from a log-log transformation plot of plasma lactate concentration vs. power output (W). Predicted $\dot{V}O_{2\text{ peak}}$ was calculated according to the formulae of Wasserman et al. ¹⁷.

Muscle Strength Testing. Unilateral (right leg) skeletal muscle strength and endurance for knee extension / flexion were assessed using an isokinetic dynamometer (MERAC[®])

(Universal, Cedar Rapids, Iowa, USA), with microprocessor, as previously described^{16,18}.

Dual Energy X-ray Absorptiometry (DEXA). 11 of the CHF patients and the 8 healthy control subjects underwent total body and thigh DEXA scanning (DXA, Lunar Corp. using DPX-L, Version 4.6E software, Madison, WI), using < 0.2 microsieverts, to determine body composition including lean tissue mass.

Muscle Biopsy. Approximately seven days after the incremental exercise test, a resting muscle biopsy was obtained from the vastus lateralis. For those on anticoagulant medications, these were discontinued for four days prior to the biopsy. Following injection of a local anaesthetic (2% Lignocaine) into the skin and against the fascia, a small incision was made with a scalpel and a muscle sample was taken with a 5mm diameter muscle biopsy needle. One portion of muscle (15-20mg) was embedded in Tissue Tek mounting medium and immediately immersed in isopentane, cooled in liquid nitrogen, and stored in liquid nitrogen for later histochemical analysis. A second piece of muscle (15-20mg) was immediately frozen in liquid nitrogen for later analyses of enzyme activities. A third piece of fresh muscle (35-40mg) was placed on ice and immediately taken to the laboratory for the determination of mitochondrial ATP production rate (MAPR).

Mitochondrial Preparation. Fresh muscle was placed on a plate over ice, dissected free of visible connective tissue, minced finely with a scalpel blade and then transferred to a

ground glass tissue homogenising tube (Kontes, New Jersey) for weighing. The muscle was homogenized over ice, with a ground glass pestle, for approximately 5 minutes. The homogenising solutions and procedures for the preparation of the mitochondrial suspension have been described by Wibom and Hultman ¹⁹.

MAPR Measurement. MAPR was determined at 25°C using the method and substrate concentrations described by Wibom and Hultman ¹⁹ in the presence of the following substrate combinations: pyruvate + malate (P+M), palmitoyl-L-carnitine + malate (PC+M), α -ketoglutarate (α -kg), succinate + rotenone (S+R) and pyruvate + palmitoyl-L-carnitine + α -ketoglutarate + malate (PPKM). ATP production by the adenylate kinase reaction and other non-specific reactions was determined from a blank containing ADP and mitochondrial suspension alone. Details of the procedure for MAPR in the presence of these substrates as used in our laboratory have been described previously ⁵ with the exception of the method used to determine mitochondrial yield which is described below. All measurements of MAPR were made in duplicate, completed within 4 h of biopsy and are expressed as $\text{mmol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ wet weight of muscle.

Measurement of Citrate Synthase activity. Citrate synthase (CS) is a specific mitochondrial marker enzyme and was used to determine mitochondrial yield in the suspensions. The activity of CS in intact mitochondria (CS_{im}) was determined from CS activity of the mitochondrial suspension before and after mitochondrial disruption. Total muscle CS activity (CS_{t}) was determined on a separate piece of muscle homogenized in 175mM KCl and 2mM EDTA (pH 7.4). All CS activity measurements were performed at

25°C with spectrophotometric detection (LKB Biochrom, Ultrospec II, Cambridge, England) in a reaction mixture containing 100mM Tris buffer (pH 8.3), 1mM DTNB, 3mM acetyl CoA and 10mM oxaloacetate according to the method of Srere²⁰. The yield of intact mitochondria in the mitochondrial suspension was calculated as the ratio of CS_{im} to CS_t.

Measurement of other Enzyme Activities. The enzyme activities of the fatty acid β -oxidative enzyme β -hydroxyacyl coenzyme A dehydrogenase (HAD) and the glycolytic enzymes phosphofructokinase (PFK) and lactate dehydrogenase (LDH), were also measured. Whole muscle homogenates (1:100 dilution) were prepared on ice in 0.17M phosphate buffer at pH 7.4 containing 0.02% bovine serum albumin (BSA) and 5mM 2-mercaptoethanol according to the method of Green et al.²¹.

PFK was assayed on fresh homogenate²¹. LDH was assayed on stored homogenate using a reaction mixture (pH 7.0) containing 100mM Imidazole, 1.0mM pyruvate, 0.04% BSA and 25 μ M NADH based on the method of Costill et al.²². HAD was assayed on stored homogenate with 150mM Imidazole-HCl buffer (pH 6.0), 1mM EDTA, 200 μ M S-acetoacetyl CoA, 0.05% BSA and 100 μ M NADH in the reaction mixture²³.

Histochemistry. For muscle fiber type determinations serial transverse sections (10 μ m), of the previously mounted muscle tissue, were cut in a cryostat (Microm, Heidelberg) at -20°C. The sections were mounted on coverslips and stained for myofibrillar ATPase at pH 9.4 following acid (pH 4.3 and 4.6) and alkaline (pH 10.35) pre-incubations to

classify type I, IIA and IIX muscle fibers as has previously been described ²⁴. Additional serial cross-sections (16 μ m) of the muscle were stained for capillary density using the periodic acid Schiff-amylase method ²⁵.

Fiber type percentages were determined from sections containing 204 ± 19 (mean \pm S.E.) fibers. Entire sections stained for capillaries were used to compute capillary to fiber ratio (203 ± 13). Mean capillary contacts per fiber type were determined on 20 contiguous fibers of each type except where there were insufficient fibers of a particular type. In this case capillary contacts for the fiber type were made on the number of fibers of that type available. Cross-sectional area measurements of specific fiber types were made using a computerized imaging system (Analytical Imaging System, Imaging Research Inc, 1999). The mean cross-sectional area (μm^2) of each fiber type was computed based on 20 contiguous fiber areas per type, as recommended by Simoneau and colleagues ²⁶.

Statistical Analysis. Data from patients and control subjects were compared using unpaired Student's *t* tests for independent variables. Data are expressed as means \pm S.E. A *p* value of less than 0.05 was considered significant. Correlations were performed using the regression function on Microsoft Excel.

RESULTS

Exercise Tolerance. Compared with their healthy counterparts CHF patients had significantly lower peak oxygen consumption ($\dot{V}\text{O}_{2\text{ peak}}$; 15.1 ± 1.1 vs. 28.1 ± 2.3 ml.kg⁻¹.min⁻¹; *p* < 0.001) (Table 2). Strength of the quadriceps (*p* < 0.05) and hamstrings (*p* <

0.01) were also lower in the CHF patient group compared to the healthy control subjects (Table 2). The CHF patient group had an earlier lactate threshold (Table 3), occurring at 30.3 ± 3.5 W versus 81.6 ± 8.6 W for control subjects ($p < 0.001$), and at termination of exercise, exhibited a lower peak work rate ($p < 0.01$). Lactate threshold when expressed as a percentage of peak work rate achieved during the exercise test was lower in the CHF group than in the normal subjects ($p < 0.01$) (Table 3). There was no difference in peak RER between the CHF patient group and the control subjects indicating that subjects ceased exercise at similar relative exercise intensities. (Table 3). No patient desaturated at the termination of exercise.

Muscle Oxidative Capacity. There was no difference in MAPR between the CHF patients and the healthy control group for any of the substrate combinations (Figure 1). The yield of intact mitochondria used for the MAPR assay was not statistically different between the CHF group (19.7 ± 2.2 %) and the healthy subjects (26.1 ± 2.4 %). There were no significant differences in the activities of the oxidative enzymes CS and HAD or in the activities of the glycolytic enzyme LDH (Table 4). A trend towards significance was observed in the glycolytic enzyme PFK ($p = 0.12$) between CHF patients and healthy controls.

Muscle Fiber Types. Histochemical data was obtained from 13 CHF patients and seven controls. CHF patients exhibited a lower proportion of type IIA muscle fibers (20.4 ± 2.1 vs. 35.3 ± 1.9 ; $p < 0.0001$) and higher proportion of type IIX muscle fibers (32.6 ± 1.8 vs. 18.5 ± 1.7 ; $p < 0.0001$) than the healthy subjects (Figure 2). No difference was found in

the proportion of type I fibers ($p = 0.65$) between the CHF and healthy subjects (Figure 2). However, type I fiber cross-sectional area (μm^2) was lower in the CHF patient group than the healthy subjects (3026 ± 175 vs. 3681 ± 210 ; $p < 0.05$). No difference between groups was found in the cross-sectional areas of either type IIA or type IIX muscle fibers.

Capillary Density. Capillary to fiber ratio was lower in CHF patients ($p < 0.001$) than in control subjects (Table 5). CHF patients exhibited fewer capillaries surrounding type I fibers ($p < 0.001$) and type IIX fibers ($p < 0.05$) than controls. A trend was observed for fewer capillaries surrounding type IIA fibers ($p = 0.075$) in CHF patients compared to healthy controls. However, when capillary density was determined per unit of muscle fiber cross-sectional area, there was no difference between CHF patients and control subjects (Table 5). There was no correlation between capillary to fiber ratio and $\dot{V}\text{O}_{2\text{ peak}}$ in either the CHF patients ($r = 0.24$; $p = 0.43$) or the healthy control group ($r = 0.14$; $p = 0.76$). Neither was there a correlation between capillary to fiber ratio and muscle strength in either the CHF patients ($r = 0.008$, $p = 0.98$) or healthy subjects ($r = 0.34$, $p = 0.50$).

DEXA. No difference was found between the CHF patients and the normal controls in either lean body mass or lean thigh mass (Table 6). There was a significant correlation between thigh lean mass and $\dot{V}\text{O}_{2\text{ peak}}$ for the CHF patients but this did not reach statistical significance in the healthy subjects (Figure 3). Additionally whole body lean mass was also significantly correlated with $\dot{V}\text{O}_{2\text{ peak}}$ ($r = 0.713$; $p < 0.05$) in CHF patients but again this did not reach significance in healthy subjects.

DISCUSSION

This is the first study to examine muscle oxidative capacity as measured by MAPR in CHF patients. The major finding of this study is that, notwithstanding a very significant difference in $\dot{V}O_{2\text{peak}}$, there is no difference in MAPR between CHF patients and similarly aged healthy individuals. Studies investigating the effects of CHF on the activity of mitochondrial enzymes in skeletal muscle have produced equivocal results. Significant reductions in the activity of citrate synthase (CS)¹⁰⁻¹², β -hydroxyacyl coenzyme A dehydrogenase (HAD)^{13,14} and succinate dehydrogenase (SDH)^{10,13} have been reported. Other studies have reported no differences in CS^{13,14,29}, HAD¹¹ and SDH²⁹. Nevertheless nuclear magnetic resonance (NMR) studies of muscle metabolism in patients affected by CHF have found more rapid depletion of phosphocreatine (PCr) during exercise^{27,28} and lower rates of PCr resynthesis²⁷ indicating reduced muscle oxidative function. Hence it is not clear which of the major metabolic pathways, if any, plays a significant role in reduced exercise tolerance in CHF patients. Therefore while oxidative function is impaired *in vivo*, the role of reduced muscle oxidative capacity as a causative factor in reduced exercise tolerance in CHF patients is less clear.

The measurement of mitochondrial enzyme activity is at best an indirect indicator of muscle oxidative capacity and only provides information about single reactions in complex pathways. In addition, maximal enzymatic activities are in general substantially in excess of *in vivo* maximal mitochondrial oxidative function³⁰. In contrast, the method of MAPR, used in the current study, is a direct and comprehensive assessment of muscle oxidative capacity across the entire range of metabolic energy producing pathways^{19,31,32}.

Previous studies have demonstrated that MAPR is strongly associated with $\dot{V}O_{2\text{ peak}}$. It is higher in endurance trained individuals compared to sedentary subjects¹⁹ and increases with endurance training^{31,32}. No studies to date have examined the effect of CHF on MAPR. Importantly, we found no difference between CHF patients and controls in MAPR in the presence of any substrate combination. Consistent with this observation we also found no difference in the activity of the mitochondrial enzymes CS and HAD. Clearly the current study is not consistent with other published literature and such differences in the data may be due in part to differences in the severity of the condition and/or the duration for which the patient has suffered CHF. The CHF patients included in this study are representative of the patients with systolic heart failure who attend a heart failure clinic, not having been selected because of cachexia or severe functional impairment, neither those referred specifically for cardiac transplantation. This is reflected in the mean age 68 ± 9 years with a mean LVEF $27\pm 8\%$ on gated blood pool scanning. Our observations demonstrate that the reduced maximal oxygen consumption in this cohort of CHF patients must be due to factors other than muscle oxidative capacity.

Muscle fiber types. Muscle biopsy studies in CHF have consistently demonstrated changes in muscle histochemistry including a reduced capillary to fiber ratio¹³ and an increase in type IIX fiber proportions at the expense of either type I^{10,33} and / or type IIA muscle fibers^{11,13}. Significant muscle fiber atrophy³³ and reduced total muscle cross sectional areas²⁷ have also been reported in CHF patients. Alterations in fiber type proportions are a potential cause of the exercise intolerance in CHF. The proportion of

type IIA muscle fibers was lower and the proportion of type IIX fibers was higher in the CHF group in the current study. Paradoxically, the increased proportion of type IIX muscle fibers did not result in a lower muscle oxidative capacity in CHF. Bigard et al.³⁴ however have demonstrated that muscle deconditioning induced by hindlimb suspension in rats results in slow to fast phenotype transitions without change in mitochondrial function. We conclude, therefore, that fiber type transformations do not necessarily result in reductions in overall muscle oxidative capacity.

Capillary density. The major function of the capillary bed in skeletal muscle is to supply oxygen and nutrients to the muscle fibers. Endurance training stimulates an increase in capillary density in human subjects without heart failure^{35,36} indicating a potential correlation between capillary density and $\dot{V}O_2$ peak. In the present study there was no correlation between $\dot{V}O_2$ peak and the capillary to muscle fiber ratio in the CHF group. This observation is supported by the findings of Duscha et al.¹³ who found no significant difference in capillary to fiber ratio between the most exercise-impaired CHF patients (< 4 METS) and the normal control group. In the current study the capillary to fiber ratio and capillary contacts per fiber were lower in the CHF group in comparison to the healthy subjects.

The current study highlights the importance of measuring capillary density as a function of muscle fiber size. When capillary density was calculated relative to fiber area (capillaries/mm²), no difference between the CHF patients and the healthy control subjects was observed. The implication of this finding is that capillary density is not a

factor in the reduced exercise tolerance in CHF patients. This finding is also supported by DeSousa and colleagues³⁷ who reported no difference in capillaries relative to fiber area between CHF and sham-operated rats in either oxidative (soleus) or glycolytic (gastrocnemius) muscles.

Role of muscle mass in reduced exercise capacity. $\dot{V}O_{2\text{ peak}}$ (ml/min) was significantly correlated with lean thigh mass in the CHF patients but less so in the normal healthy controls (Figure 3). This relationship in CHF patients has previously been observed in larger patient groups.^{38,39} This suggests that reduced muscle mass could be a determinant of the reduced exercise capacity in CHF. Although the mean lean thigh and lean body mass were lower in the CHF group compared to the healthy subjects, these differences did not reach statistical significance. Nevertheless, the CHF patients did have significantly smaller type I muscle fibers, a lower lean thigh mass to body mass ratio and reduced thigh muscle strength (Table 2). Previous studies using computerized tomography³⁹ and magnetic resonance imaging^{27,40} have demonstrated smaller muscle cross sectional areas in CHF patients. The reduction in the cross-sectional area of the type I fibers suggests that the CHF patients might recruit type II muscle fibers at lower exercise intensities. Additionally, the CHF group had a significantly lower proportion of type IIA fibers indicating an earlier reliance on glycolytic type IIX fibers during the incremental exercise test. This conclusion is substantiated by the earlier onset of lactic acidosis in the CHF group.

While muscle mass might be contributing to the difference in exercise capacity between the groups, the difference in lean body mass is unlikely to account for all of the substantially lower $\dot{V}O_{2\text{ peak}}$ in the CHF patients compared to the healthy control subjects.

Metabolic control. While our data demonstrate that skeletal muscle oxidative capacity is not reduced in our cohort of CHF patients it is possible that the rate of oxidative phosphorylation during exercise is reduced. *In vivo* studies using P-31 nuclear magnetic resonance (P-31 NMR) spectroscopy have demonstrated reduced phosphocreatine (PCr) resynthesis post-exercise in CHF patients^{27,41}. As PCr resynthesis is an oxidative process, this suggests an impaired rate of oxidative phosphorylation. Cohen-Solal et al.⁴¹ also reported slower kinetics of recovery of oxygen consumption in CHF patients after exercise, further supporting an impaired rate of muscle oxidative metabolism. Additionally, the PCr concentration in muscle declines more rapidly during submaximal exercise in CHF patients indicating lower rates of oxidative phosphorylation for given absolute and relative submaximal exercise loads^{6,42}. Skeletal muscle pH also began to decline earlier during exercise indicating an earlier onset of glycolysis. This is consistent with the earlier onset of lactic acidosis which was observed in the current study. Clearly, whilst we provide evidence that skeletal muscle oxidative capacity is not reduced, oxidative phosphorylation during exercise is impaired in CHF.

A number of factors may be responsible for reducing oxidative phosphorylation during exercise in CHF. Toussaint et al.⁴³ have shown reduced reactive hyperemic flow in the calf muscles of CHF patients along with slowed PCr recovery. The authors concluded

that oxidative ATP resynthesis is limited by reduced oxygen availability, the result of impaired blood flow.

In oxidative, but not glycolytic muscle, elevated levels of creatine considerably enhance respiratory control by ADP. Hence, during exercise, mitochondrial respiration is stimulated by increased cytosolic creatine levels in oxidative but not glycolytic muscles. Consequently, fiber type alterations that increase glycolytic fiber proportions would reduce the sensitivity of whole muscle oxidative phosphorylation to ADP stimulation. The higher proportion of glycolytic fibers in CHF patients than in controls may explain the aforementioned lower levels of oxidative phosphorylation during exercise.³⁷ A further mechanism related to the control of oxidative phosphorylation during exercise, as described by Conley et al.,⁴⁴ may also explain the lower exercise capacity of CHF patients. During exercise PCr levels fall and ADP levels rise as described by the creatine kinase equilibrium shown below



The ADP level is the central signal for oxidative phosphorylation. Conley et al.⁴⁴ point out that the rise in ADP as PCr levels fall is attenuated at lower intramuscular pH. In our CHF group we observed higher proportions of type IIX glycolytic muscle fibers and an earlier onset of lactic acidosis in incremental exercise potentially reflecting a lower muscle pH. We postulate, therefore, that the rate of rise of ADP in the CHF group and the rate of oxidative phosphorylation during incremental exercise would be less than that in

the controls leading to a greater reliance on glycolytic ATP production. In support of this claim Massie et al. ⁶ have observed a lower muscle pH during submaximal exercise in CHF patients compared to healthy control subjects.

Conclusions. The major finding of this study was that there was no difference between CHF patients and healthy control subjects in skeletal muscle MAPR across the full range of substrates. Neither was there impaired activity of the muscle oxidative enzymes investigated. Therefore the CHF patients in this study, who are generally older and perhaps more representative of CHF patients in the general community than those described in previous studies, appear to have normal resting skeletal muscle oxidative function, although impaired oxidative phosphorylation during exercise cannot be excluded.

Capillary density is reduced in these CHF patients in association with muscle fiber atrophy. However, the reduced $\dot{V}O_{2\text{ peak}}$ does not appear to be associated with this reduction in capillary density.

Lower lean body mass and type I fiber atrophy are both associated with the impairment in $\dot{V}O_{2\text{ peak}}$. It is unclear whether or not these morphological changes in skeletal muscle might be reversible following muscle-strengthening exercise training and/or better treatment of the underlying heart failure.

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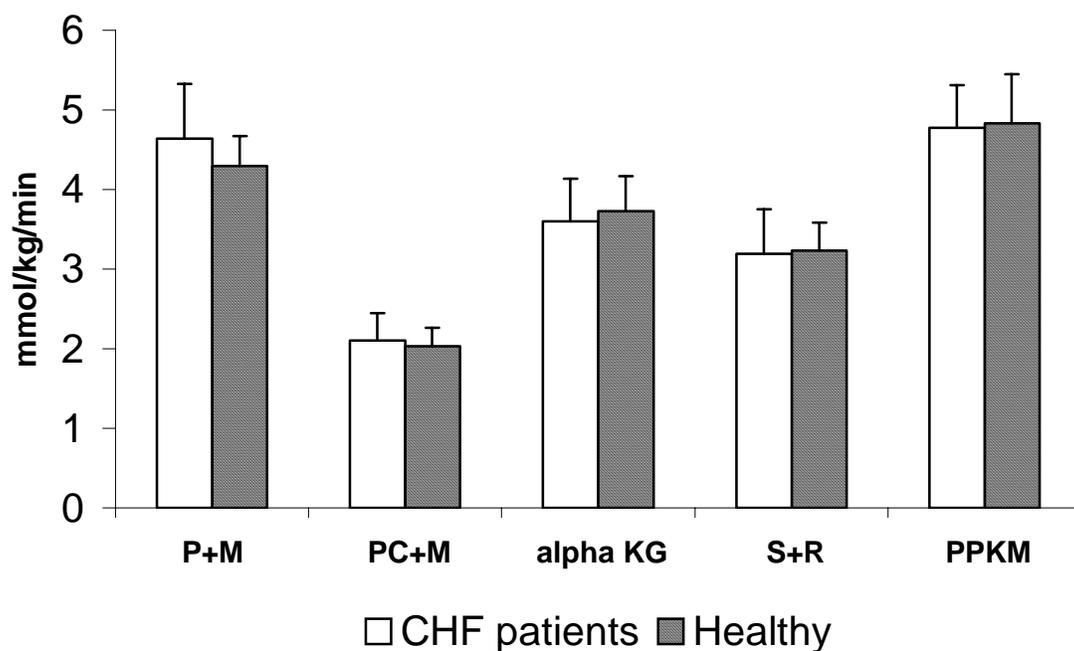


Figure 1. Mitochondrial ATP Production Rate in Patients With Class II-III CHF and Healthy Subjects (Mean \pm SEM). The substrate combinations were: P+M = pyruvate + malate; PC+M = palmitoyl-L-carnitine + malate; α -KG = α -ketoglutarate; S+R = succinate + rotenone; PPKM = pyruvate + palmitoyl-L-carnitine + α -ketoglutarate + malate. CHF = chronic heart failure.

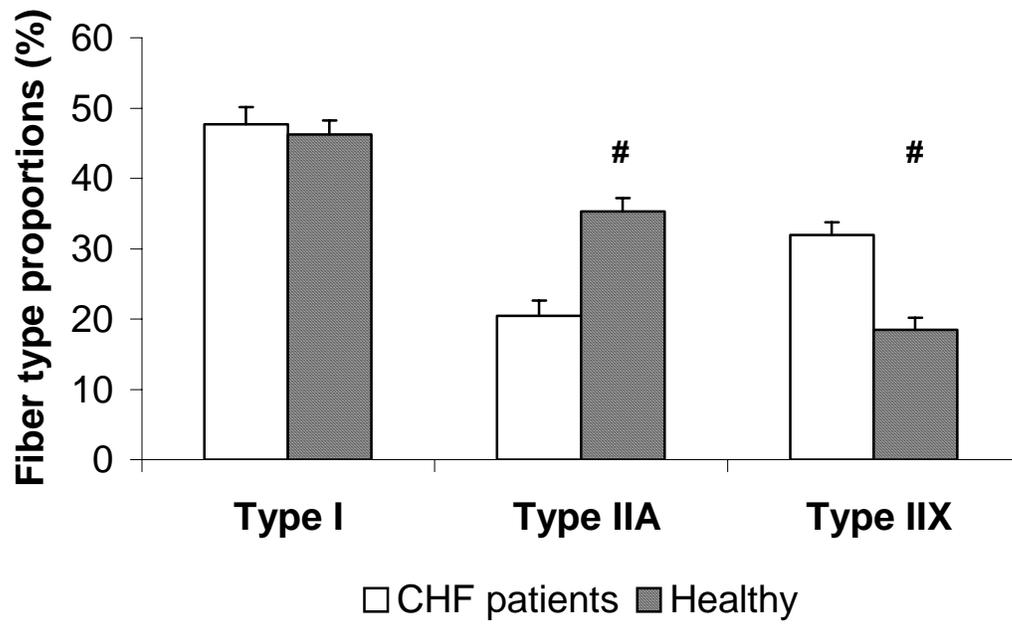


Figure 2. Skeletal Muscle Fiber Type Proportions in CHF Patients and Healthy Subjects

[#] denotes $p < 0.0001$. CHF = chronic heart failure.

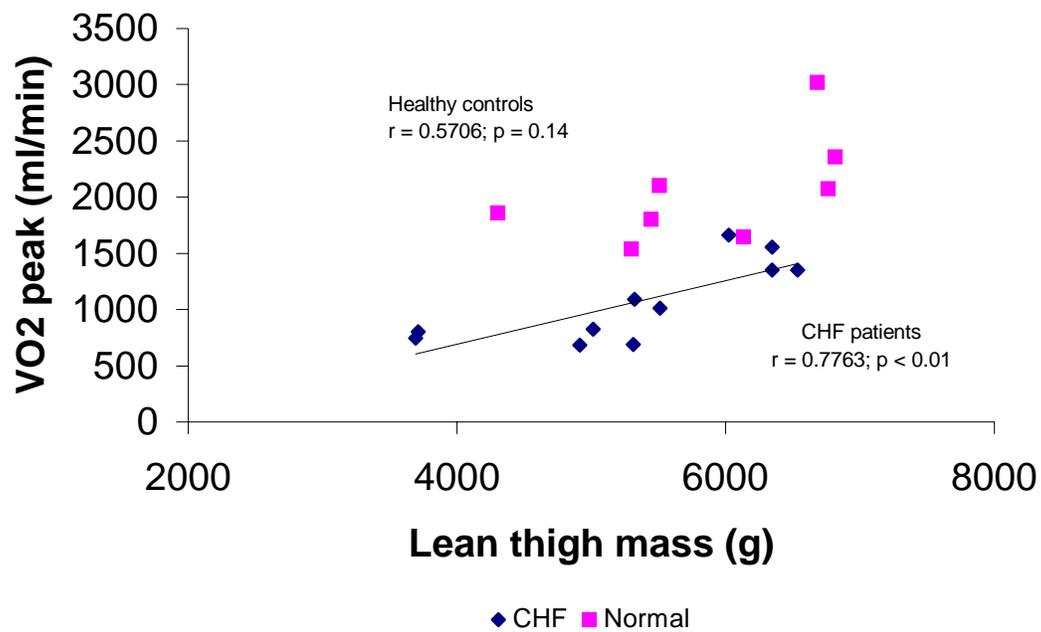


Figure 3. Correlations between Lean Thigh Mass and VO₂ peak for 11 CHF and 8 Healthy Controls. CHF = chronic heart failure.

Table 1. Descriptive characteristics of the 14 CHF patients (Mean \pm S.D.)

| CHF patients (n=14) | |
|---|----------|
| CHF diagnosis | |
| Ischemic heart disease | 8 (57%) |
| Dilated cardiomyopathy | 5 (36%) |
| Valvular | 1 (7%) |
| Medications | |
| Angiotensin converting enzyme inhibitor | 11 (79%) |
| Angiotensin receptor blocker | 3 (21%) |
| Diuretic | 13 (93%) |
| Beta-blocker | 6 (43%) |
| Digoxin | 6 (43%) |
| Aspirin | 11 (79%) |
| Warfarin | 4 (29%) |
| Amiodarone | 2 (14%) |
| Nitrates | 7 (50%) |
| Calcium channel antagonist | 2 (14%) |

Table 2. Skeletal muscle strength and $\dot{V}O_2$ peak data for CHF patients and Healthy subjects

| | CHF | Healthy |
|---|----------------|-----------------------------|
| $\dot{V}O_2$ peak (ml.kg ⁻¹ .min ⁻¹) | 15.1 \pm 1.1 | 28.1 \pm 2.3 [‡] |
| % Predicted $\dot{V}O_2$ peak | 64 \pm 4 | 111 \pm 9 [‡] |
| Knee Extension (Nm) | 105 \pm 11 | 133 \pm 6* |
| Knee Flexion (Nm) | 45 \pm 5 | 64 \pm 4 [†] |

*Denotes $p < 0.05$. [†]Denotes $p < 0.01$. [‡]Denotes $p < 0.001$.

Table 3. Plasma Lactate and Workload Data for CHF Patients and Healthy Subjects

| | CHF | Healthy |
|---------------------------------------|-------------|-------------------------|
| Peak Watts | 64 ± 7 | 134 ± 14 [†] |
| Lactate at fatigue (mmol/L) | 4.6 ± 0.6 | 8.2 ± 0.9 [†] |
| Lactate Threshold (W) | 30.3 ± 3.5 | 81.6 ± 8.6 [‡] |
| Lactate Threshold/ Peak Watts (%) | 49.1 ± 2.7 | 61.7 ± 3.3 [†] |
| Peak Respiratory Exchange Ratio (RER) | 1.12 ± 0.04 | 1.16 ± 0.03 |

[†]Denotes $p < 0.01$. [‡]Denotes $p < 0.001$.

Table 4. Skeletal Muscle Enzyme Activity in Patients With Class II-III CHF and Healthy Subjects ($\mu\text{mol}/\text{min}/\text{g}$ WW)

| <i>Enzyme</i> | CHF | Healthy |
|--|--------------|----------------|
| Citrate Synthase | 14.43 ± 1.02 | 14.63 ± 0.85 |
| β -Hydroxyacyl-CoA Dehydrogenase | 13.06 ± 0.89 | 13.58 ± 1.25 |
| Phosphofructokinase | 29.80 ± 1.17 | 27.47 ± 1.13 |
| Lactate Dehydrogenase | 60.29 ± 8.89 | 79.75 ± 13.69 |

Table 5. Skeletal Muscle Capillary Density in CHF Patients and Healthy Subjects

| | CHF | Healthy (n=7) |
|---|-------------|--------------------------|
| Capillary to Fiber Ratio | 1.09 ± 0.05 | 1.40 ± 0.04 [†] |
| Capillaries Contacting Individual Fibers | | |
| Type I | 3.02 ± 0.13 | 3.82 ± 0.11 [†] |
| Type IIA | 2.71 ± 0.15 | 3.06 ± 0.10 |
| Type IIB | 2.14 ± 0.11 | 2.46 ± 0.08 [*] |
| Capillaries per mm ² of fiber area | | |
| Type I | 1147 ± 85 | 1122 ± 87 |
| Type IIA | 1038 ± 45 | 1082 ± 65 |
| Type IIB | 1017 ± 58 | 1118 ± 100 |

*Denotes p < 0.05. [†]Denotes p < 0.001.

Table 6. Total Body and Thigh Lean Masses and Lean Thigh/Total Body Mass Ratio in CHF Patients (n = 11) and Healthy Subjects

| | CHF | Healthy |
|------------------------|--------------|--------------------------|
| Lean Thigh Mass (kg) | 5.34 ± 0.30 | 5.87 ± 0.31 |
| Lean Body Mass (kg) | 50.79 ± 2.31 | 52.83 ± 2.43 |
| Lean Thigh/Body Mass % | 6.97 ± 0.24 | 8.01 ± 0.34 [*] |

*Denotes p < 0.05.