SKELETAL MUSCLE STRUCTURE AND METABOLISM IN CHRONIC HEART FAILURE

By

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TABLE OF CONTENTS

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DECLARATION

This dissertation summarises original work conducted in the School of Biomedical Science at Victoria University of Technology except where acknowledged otherwise.

This thesis is the result of work performed by the author with the following exceptions. Due to the complexity of the studies and the invasive procedures undertaken, collaboration for this research was considerable. In addition, the training study described in this thesis formed part of a larger more expansive study involving other postgraduate students. The author had a large role in the supervision of exercise testing and training sessions in the larger study. Medical personnel conducted the muscle biopsies and recruited the chronic heart failure patients. Ms. Deidre Toia assisted with the organisation and supervison of subjects for the experimental trials and training sessions. Dr. Steve Selig, Mr. David Menzies, Mr. Nathan Butler and Mr Jeremy Patterson assisted in the conduct of the exercise tests and training sessions. $\dot{V}O_{2\,peak}$, peak power and muscle strength data from the full cohort of patients in the resistance training study have been used by other postgraduate students.

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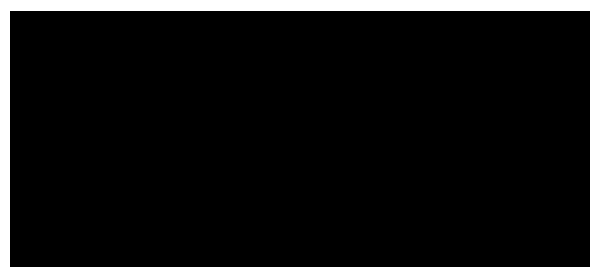


Andrew Dafydd Williams

ABSTRACT

Chronic heart failure (CHF) patients have poor exercise tolerance, which does not correlate with impaired central function. Consequently factors other than impaired cardiac function must contribute to the exercise intolerance. Maladaptations in skeletal muscle appear to be a likely reason for the limited exercise tolerance. In study I (Chapter 4), exercise tolerance and skeletal muscle metabolism and morphology were examined in 17 CHF patients and eight healthy similarly aged sedentary control subjects. $\dot{V}O_{2peak}$, peak work rate, lactate threshold and muscle strength were all lower in the CHF patients compared to the healthy control subjects. CHF patients exhibited a lower proportion of type IIA ($p < 0.0001$) and higher proportion of type IIX muscle fibres ($p < 0.0001$), and type I muscle fibres of the CHF patients were smaller than those of the healthy control subjects ($p < 0.05$). No differences were noted oxidative enzymes or mitochondrial ATP production rates which are indices of muscle oxidative capacity. Consequently muscle wasting appears to contribute to the exercise intolerance experienced with heart failure although a role for impaired oxidative function *in vivo* cannot be excluded.

In Study II (Chapter 5), thirteen CHF patients participated in a study on the effects of resistance training on exercise tolerance and skeletal muscle morphology and metabolism. Subjects were randomized either to 11 weeks of resistance training (n=7) or to maintenance of existing activity levels (n=6). Resistance training resulted in improvements in $\dot{V}O_{2peak}$ ($p<0.05$), lactate threshold ($p<0.05$), muscle strength ($p<0.05$) and several indices of muscle oxidative capacity ($p<0.05$). Changes in muscle oxidative capacity were strongly correlated to changes in $\dot{V}O_{2peak}$ ($p<0.0001$) over the 11-week intervention. However no alterations were noted in skeletal muscle fibre proportions, size or capillary density. While these results indicate a beneficial effect of

resistance training on exercise capacity in CHF they were due to improvements in muscle oxidative function (as indicated by the increase in lactate threshold and increased oxidative capacity) rather than alterations in muscle morphology.

Tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS) and elevations in the ratio of cortisol to testosterone have all been implicated as possible factors involved in muscle wasting in CHF. To determine the role of these signaling molecules on exercise tolerance and muscle oxidative capacity and morphology in CHF they were compared in 20 CHF patients and eight healthy control subjects (Chapter 6). Changes in these variables with resistance training were also compared in a group of CHF patients. There was a trend towards TNF-α being significantly different between the CHF patients and healthy control subjects ($p = 0.09$). However there was only a weak relationship between TNF-α and exercise tolerance ($p = 0.17$) and no change in TNF-α with training. No differences were noted in any of the other signaling molecules measured. These results contrast with those of previous studies and suggest a limited role for these compounds in the muscle maladaptations observed in chapter 4. However the lack of difference may be due to limited sample size or patient prognosis.

The Angiotensin Converting Enzyme (ACE) gene has been considered as a factor that may influence the risk of heart disease. In addition it has been proposed that the response to various forms of exercise training may be ACE genotype dependent. As a consequence the relationship between ACE genotype and exercise tolerance was determined in a group of 38 CHF patients (Chapter 7). Patients who were homozygous for the I allele were found to have an elevated $\dot{V}O_{2peak}$ ($p < 0.05$) and elevated muscle oxidative capacity ($p < 0.05$) compared to patients with

the D allele. When gender differences between the groups were taken into account the significance in $\dot{V}O_{2peak}$ disappeared although trends were still apparent. In subjects who volunteered for a muscle biopsy the D allele was associated with lower mitochondrial ATP production rates ($p<0.05$) than those homozygous for the I allele. A subgroup of the patients undertook eleven weeks of resistance training (n=19). $\dot{V}O_{2peak}$ increased significantly as a result of resistance training in subjects with the D allele ($p<0.05$) but did not change in subjects homozygous for the I allele. ACE genotype therefore, may have a role in exercise tolerance in CHF and may influence the effectiveness of resistance training in this condition.

ABBREVIATIONS

α-KG ..... Alpha ketoglutarate

ACE ..... Angiotensin converting enzyme

ADP ..... Adenosine diphosphate

AMP ..... Adenosine monophosphate

ANT ..... Adenine nucleotide translocase

ATP ..... Adenosine triphosphate

BMI ..... Body mass index

CAD ..... Coronary artery disease

cGMP ..... Cyclic guanosine monophosphate

CHF ..... Chronic heart failure

COPD ..... Chronic obstructive pulmonary disease

COX ..... Cytochrome-c-oxidase

CPT I ..... Carnitine palmitoyltransferase I

CPT II ..... Carnitine palmitoyltransferase II

CS ..... Citrate synthase

CSA ..... Cross sectional area

DCM ..... Dilated cardiomyopathy

dH_2O ..... Distilled water

e^- ..... Electron

EDL ..... Extensor digitorum longus

eNOS ..... Endothelial nitric oxide synthase

ETC ..... Electron transport chain

FAD..... Flavin adenine dinucleotide

FFA.....Free fatty acid

GAPDH..... Glyceraldehyde-3-phosphate dehydrogenase

GDH.....Glutamate dehydrogenase

H^+..... Hydrogen ion

HAD.....Beta-hydroxyacyl coenzyme A dehydrogenase

IFN-γ.....Interferon - γ

IGF-1.....Insulin like growth factor 1

IHD..... Ischaemic heart disease

IMP..... Inosine monophosphate

iNOS..... Inducible nitric oxide synthase

KGDH..... Alpha keto glutarate dehydrogenase

K_m.....Michaelis constant

LVEF.....Left ventricular ejection fraction

MAPR..... Mitochondrial ATP production rate

MDH..... Malate dehydrogenase

MHC.....Myosin heavy chain

MI..... Myocardial infarction

MVC.....Maximal voluntary contraction

Myosin (HE).....Myosin in its energized state

Myosin (LE)..... Myosin in its low energy state

NAD^+.....Nicotinamide adenine dinucleotide

NF-κB.....Nuclear factor-κB

NMRS ..... Nuclear magnetic resonance spectroscopy

nNOS ..... Neuronal nitric oxide synthase

NO ..... Nitric oxide

NYHA ..... New York Heart Association

P&M ..... Pyruvate and Malate

PC&M ..... Palmitoyl–L–Carnitine and Malate

PCr ..... Phosphocreatine

PFK ..... Phosphofructokinase

PHOS ..... Glycogen phosphorylase

P*i* ..... Inorganic phosphate

PPKM ..... Pyruvate and Palmitoyl-L-Carnitine and Alpha-ketoglutarate and Malate

RAS ..... Renin – Angiotensin system

RER ..... Respiratory Exchange Ratio

RM ..... Repetition maximum

ROS ..... Reactive oxygen species

RT ..... Resistance training

S&R ..... Succinate and Rotenone

SCR ..... Succinate cytochrome reductase

SDH ..... Succinate dehydrogenase

SR ..... Sarcoplasmic reticulum

TNF-α ..... Tumor Necrosis Factor - α

UQ ..... Ubiquinone

$\mathbf{V_{max}}$ ..... Maximal velocity of shortening of skeletal muscle

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER 1. INTRODUCTION

Traditionally people diagnosed with cardiac failure have been advised to rest and not perform any strenuous exercise. It is now widely recognised however that this style of treatment only exacerbates the low exercise tolerance that is seen in this syndrome and which is strongly related to a negative prognosis. In the last two decades there has been much research published investigating the possible mechanisms responsible for low exercise tolerance in chronic heart failure (CHF) patients. While it may be expected that the exercise intolerance in CHF would be related to impairment in central function the results of multiple studies have suggested that this is not the case (Joundeau et al. 1992; Cohn et al. 1993).

With the research demonstrating that central mechanisms alone do not account for the reduced exercise tolerance in CHF patients, the focus has shifted to the role of skeletal muscle maladaptations. A number of studies have investigated the effect of CHF on oxidative function in CHF with mixed results. Several studies have examined the effects of CHF on oxidative phosphorylation *in vivo* and reported reduced rates of oxidative phosphorylation in CHF patients (Massie et al. 1988; Mancini et al. 1992). However these studies were limited in that the exercise was limited to small muscle groups preventing a maximal systemic response from occurring. Other investigators have chosen to investigate the effect of CHF on oxidative capacity *in vitro*. Sullivan et al. (1990) reported reductions in both citrate synthase (CS) and succinate dehydrogenase (SDH) activity in CHF patients while Opasich et al. (1996) reported no difference in either of these enzymes in their CHF group. Similar studies have reported significant reductions in some but not all oxidative enzymes measured (Duscha et al. 1999; Duscha et al. 2001; Schaufelberger et al. 2001). The only study to directly measure mitochondrial oxygen consumption in CHF reported no difference when compared to sedentary control subjects (Mettauer et al. 2001). CHF has also

been reported to result in alterations in muscle fibre type proportions (Sullivan et al. 1990; Massie et al. 1996; Duscha et al. 2002), capillary density (Sullivan et al. 1990; Duscha et al. 1999) and muscle atrophy (Harrington et al. 1997; Cicoira et al. 2001b). However the findings have not been consistent across all studies. Consequently a study designed to examine a comprehensive range of skeletal muscle variables in CHF is warranted.

Resistance training enhances muscle mass and strength in healthy young subjects (MacDougall et al. 1980). The increase in muscle mass is due to hypertrophy of the muscle fibres rather than increases in fibre number (Narici et al. 1989; Wang et al. 1993; Green et al. 1998). In addition to hypertrophy, resistance training results in reductions in the percentage of the glycolytic type IIX muscle fibres accompanied by increases in the percentage of the oxidative/glycolytic type IIA muscle fibres (Green et al. 1998; Hikida et al. 2000). In healthy elderly subjects resistance training has also been reported to result in muscle increases in oxidative enzyme activity (Frontera et al. 1990), mitochondrial volume density (Jubrias et al. 2001) and capillary supply (Hepple et al. 1997; Hagerman et al. 2000). Subjects who have undergone periods of bed rest (Ferrando et al. 1997) or muscle immobilisation (MacDougall et al. 1980) have experienced positive effects from resistance training. More recently resistance training has been demonstrated to be a safe and effective mode of exercise for patients with cardiovascular disease (Magnusson et al. 1996; McCartney 1998) and CHF (Hare et al. 1999; Pu et al. 2001). To date no studies have examined the effect of resistance training on a wide range of muscle variables in patients with CHF.

While it has been established that CHF results in maladaptations in skeletal muscle the mechanisms responsible for these changes are not well understood. The cytokine tumor necrosis factor-α (TNF-α), which has been reported to be elevated in CHF (Levine et al.

1990; Anker et al. 1997b), appears to be involved in myocardial remodelling in CHF. Elevated systemic concentrations of this cytokine may negatively impair skeletal muscle protein balance by a combination of direct (Sen et al. 1997; Li and Reid 2000; Langen et al. 2001; Li et al. 2003) and indirect (Kelly et al. 1996; Anker et al. 1997a; Fernandez-Celemin et al. 2002) mechanisms. Indirect methods of TNF-α action may affect muscle protein balance by elevating circulating cortisol (Anker et al. 1997a) or increasing inducible nitric oxide synthase (iNOS) expression (Kelly et al. 1996). Consequently TNF-α is accepted as having a role in skeletal muscle wasting in CHF. However further work is required to elucidate the exact mechanisms of action of this cytokine and other mediators of catabolism in CHF. Further as resistance training has been demonstrated to have a powerful anabolic effect in healthy subjects it is of interest to investigate the effects of resistance training in CHF with reference to its effects on mediators of anabolic/catabolic activity.

Angiotensin converting enzyme (ACE) catalyses the production of angiotensin II and renders bradykinin inactive. A polymorphism of the ACE gene in intron 16 is characterised by the presence (insertion – I) or absence (deletion – D) of a 287 base pair (bp) sequence. Previous studies have reported that the D allele of the ACE gene may be a risk factor for heart disease (Cambien et al. 1992; Ruiz et al. 1994). ACE genotype has also been related to endurance exercise capacity in healthy individuals (Hagberg et al. 1998; Montgomery et al. 1998) with the incidence of the I allele being more prevalent amongst those British Olympic standard athletes competing in longer distance running events (Myerson et al. 1999). These findings may be explained by the observations of Zhang et al. (2003) who reported a higher incidence of and type I (oxidative) muscle fibre proportions in subjects homozygous for the I allele. A single study has examined the relationship between ACE genotype and exercise tolerance in CHF patients (Abraham et al. 2002). The authors reported the DD genotype to be associated

with decreased exercise tolerance in CHF patients. However this study did not examine any potential links between the ACE genotype and muscle variables or the response to exercise training in CHF. Consequently it is of interest to determine the relationship between ACE genotype and skeletal muscle in CHF and to determine the effect of ACE genotype on the response to exercise training in CHF patients.

The general aim of this thesis is to elucidate the maladaptations that occur in the skeletal muscle of CHF patients and their relationship to the exercise intolerance that is endemic in this group. The effect of resistance training on skeletal muscle morphology and oxidative capacity in CHF is also examined as it may assist in the design of rehabilitation programs for CHF patients. The thesis also examines some of the factors considered likely to be responsible for the skeletal muscle maladaptations in CHF. The effects of resistance training on these factors are examined in order to determine their role in reduced exercise tolerance and changes in skeletal muscle parameters in CHF patients. Accordingly this thesis will examine:

1. Muscle oxidative capacity and morphology in a group of CHF patients compared to healthy sedentary age matched controls (chapter 4).
2. The effects of resistance training on exercise tolerance and skeletal muscle oxidative capacity and morphology in CHF patients (chapter 5).
3. Potential mechanisms mediating exercise tolerance and skeletal muscle parameters in chronic heart failure (chapter 6).
4. The effect of ACE genotype on exercise tolerance and response to resistance training in chronic heart failure (chapter 7).

CHAPTER 2. LITERATURE REVIEW

2.1 OVERVIEW

ATP is a molecule that is used for storage and transport of energy in all living cells. It can be referred to as the universal energy donor because it couples the energy released from the oxidation of foodstuffs into a form of energy usable by all cells. Skeletal muscle has a very high metabolic range and requires large supplies of ATP during contraction. ATP is hydrolysed to ADP in a process in which chemical energy is converted to mechanical energy. The resynthesis of ATP (to allow mechanical work to continue) relies upon a range of processes of energy metabolism. These processes can be divided into those requiring oxygen (aerobic) and those where oxygen is not required (anaerobic) for ATP resynthesis to occur. Aerobic metabolism takes place in the mitochondria, and in skeletal muscle the capacity of the mitochondria to resynthesise ATP can be affected by a range of factors. Among these factors are gender, age, physical activity levels and some disease states. Patients with chronic heart failure (CHF) have a reduced exercise capacity that has been associated with muscle fibre type alterations, low mitochondrial capacity, reduced capillary density and skeletal muscle atrophy. While significant work has been performed investigating the role of skeletal muscle maladaptations in CHF and their role in the reduced exercise tolerance, many inconsistencies exist in the literature.

The following review will discuss:

- Skeletal muscle structure
- Skeletal muscle metabolism
- Mitochondrial structure and function
- Factors affecting skeletal muscle structure and metabolism
- CHF, exercise limitation and potential causes

- Potential methods of reversing exercise intolerance in CHF

2.2 SKELETAL MUSCLE STRUCTURE

In all animals, the ability to move is essential for survival. Muscles are the contraction specialists of the body. They attach to the skeleton and during contraction apply a force to the long bones causing these bones to act as levers and initiate movement. For this to occur, the cytoplasm of skeletal muscle fibres is packed with contractile and metabolic apparatus, with the contractile apparatus accounting for approximately 75% of the volume of the fibre (Luff and Atwood 1971).

2.2.1 Muscle Fibre Sizes

The diameter of skeletal muscle fibres is generally in the range of 10 to 100μm. Different muscles have different characteristics depending on their physiological function. The tensor tympani, which is involved in adjusting tension on the eardrum contains only a few hundred muscle fibres, while the medial gastrocnemius has been reported to contain approximately one million fibres (Feinstein et al. 1955). While the size of the muscle and the orientation of individual fibres within the muscle will influence its contractile properties, other factors involved with the structure of the fibres that make up the muscle will also affect contractile force.

2.2.2 Skeletal Muscle Fibre Classifications

Skeletal muscle cells can be divided into different groups, depending on their contractile and metabolic properties. Evidence of this was reported as early as 1873 with Ranvier (1873) observing that some muscles contracted more slowly than others and had a more pronounced red colouration. It is now known that the red colour of these muscles is due to the increased

concentration of myoglobin and other iron containing compounds in the muscle fibres and that in mammals, these red fibres are more common in muscles used in the maintenance of posture. In contrast the muscles consisting predominantly of pale muscle fibres are employed intermittently in non-repetitive movements. The muscles of smaller mammals, such as rodents consist predominantly of red or white fibres. Where a muscle contains both types of fibres, they tend to be separated into pale and red parts. An example of this is the gastrocnemius of the common rat *(Rattus Norvegicus)*, where the deeper portion of the muscle is composed mainly of red fibres while the superficial part is composed primarily of pale fibres. In humans there is also a mixture of fibres within any given muscle. However, in humans the red and pale muscle fibres are mixed together to form a mosaic. The red and pale muscle fibres can be further categorised according to their metabolic, contractile and structural characteristics which can apply to whole muscles and also to single fibres. In the following sections, the different methods of categorising skeletal muscle fibres will be described and the advantages and disadvantages of these methods will be presented.

2.2.3 Contractile Characteristics of Skeletal Muscle

The identification of different types of muscle fibres on the basis of their contractile characteristics became possible following the demonstration of Kugelberg and associates (Edstrom and Kugelberg 1968; Kugelberg 1973; Kugelberg 1976) that individual motor neurons could be stimulated and that the contractile properties of fibres in these motor units was similar. Burke et al. (1971) classified the motor units of the cat gastrocnemius muscle into types based on their contractile characteristics (either slow [S] or fast [F]) and resistance to fatigue (easily fatigued [F] or fatigue resistant [R]). Three basic classes of motor units were identified using this system; slow [S] motor units which are highly resistant to fatigue; fast fatigable [FF] and fast fatigue resistant [FR]. Improved contractile function methods

mean that it is now possible to examine the contractile characteristics of single fibres that are directly stimulated rather than to have to make inferences based on the stimulation of a motor neuron. Ruff and Whittlesey (1991) used human muscle fibres taken from the lateral gastrocnemius or intercostal muscles of adult males and investigated their contractile velocities and force characteristics. Fibres were divided into three groups depending on histochemical parameters. The authors reported contraction velocities more than two fold greater in muscle fibres histochemically determined to be either type IIA or IIX fibres than in those determined to be type I fibres. When normalised for cross-sectional fibre area however, there was no difference in the maximum tension of muscle fibres between any of the fibre types, a finding that has been reported many times in a range of mammalian species (Donaldson 1984; Fink et al. 1986; Eddinger and Moss 1987; Fink et al. 1990).

2.2.4 Metabolic Characteristics of Skeletal Muscle.

Skeletal muscle can be sorted according to different metabolic characteristics of different muscles as well as of individual fibres within the muscles themselves. Muscle is most commonly sorted according to its metabolic characteristics using histochemical methods. The stains that are used are intended to reflect the activities of glycolysis, the Krebs cycle or oxidative phosphorylation in individual muscle fibres. Methods designed to rate the oxidative potential of a muscle fibre involve stains for enzymes found in the Krebs cycle or the electron transport chain (succinate dehydrogenase, cytochrome-c-oxidase, malate dehydrogenase and reduced nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR). A comparison of these methods suggests that there is little difference in the classification of fibres when using a variety of enzymes or cofactors involved in oxidative metabolism (Meijer and Elias 1976). Histochemical stains have also been employed to determine the glycolytic potential of different muscle fibres using α-glycerophosphate

dehydrogenase, lactate dehydrogenase and phosphorylase, although these methods tend to correlate poorly with findings from biochemical determinations of these enzymes in muscles containing a high proportion of the fibres evaluated (Baldwin et al. 1973). A reason for this may be due to a leaching of the enzymes into the aqueous medium during incubation and staining (Gollnick and Armstrong 1976). These methods of characterising muscle are simple and allow the identification of low and high oxidative fibres in mixed muscle samples; however, there are a number of disadvantages with fibre typing based on this method alone. A major problem with the identification of fibres based on these methods is that a continuum of oxidative potentials exists within the fibres of a given skeletal muscle (Kugelberg and Lindegren 1979; Pette and Spamer 1979) making it difficult to divide fibres into specific groupings. The intensity of a stain is also influenced by such factors as the thickness of the section and incubation time (Halkjaer-Kristensen and Ingemann-Hansen 1979). These issues make it impossible to accurately compare the metabolic characteristics of muscle fibres from different samples thereby invalidating these as creditable methods for between sample comparisons. Attempts to use histochemical stains for oxidative or glycolytic enzymes to classify fibres in other ways (eg. slow or fast twitch) cannot realistically be performed, as stains generally do not clearly differentiate fibres into groups with regard to their oxidative potential. Furthermore, attempting to predict the tension characteristics of fibres from this method is invalid as highly oxidative fibres may have either fast or slow twitch characteristics and the oxidative capacity of muscle is largely a function of its activity level as alterations in oxidative capacity are induced more readily than changes in contractile properties (Gollnick and Hodgson 1986). Consequently, while histochemical staining for the presence of muscle enzymes or cofactors can provide information regarding the metabolic properties of skeletal muscle fibres, the information gained is largely qualitative and provides little information regarding the contractile characteristics of muscle fibres.

2.2.5 Muscle Fibre Classification by Myosin Type

Muscle fibres can be classified by differences in the sensitivity of their myofibrillar myosin ATPase to either acid or alkaline pre-incubation (Brooke and Kaiser 1969; Brooke and Kaiser 1970) or differences in the molecular weight of the myosin protein (Perrie and Bumford 1984; Perrie and Bumford 1986). The myosin ATPase method is based on the sensitivity of the myofibrillar myosin ATPase to inactivation in response to acid or alkali pre-treatment (Brooke and Kaiser 1969; Brooke and Kaiser 1970). On the basis of this method, human and other mammalian muscle fibres can be characterised by the range of lability of their myosin ATPase (Table 2.1).

Table 2.1: pH stability range of myosin ATPase for different human fibre type populations.

Muscle Fibre Type	pH stability range of the myosin ATPase
Type I	4.0 – 9.5
Type IIA	4.6 – 10.5
Type IIX	5.0 – 10.5

A more recent method of muscle fibre classification is by the identification of different molecular forms of key muscle proteins. Specific antibodies can be produced for selected proteins and by exposing muscle sections to these antibodies it is possible to identify specific proteins in the muscle and to classify the muscle on the basis of the proteins that are present. Myosin has been most extensively studied in this way. In particular, the proteins that make up the myosin heavy chains (MHC), (each myosin consists of two heavy and four light chains), and 10 different isoforms have been identified in mammalian muscle (Kelly and Rubinstein 1994). However, a number of these isoforms appear to be either developmental or found only in specialised muscles (Draeger et al. 1987). The major fibre classes identified in mammals through analysis of MHC are I, IIA, IIB and IIX. However, it is now known that

the third type II fibre population reported in the skeletal muscles of rats and identified as type IIX has the same pattern of expression and sequence homology as those previously identified as type IIB in humans (Smerdu et al. 1994). Consequently this thesis will describe human muscle fibres identified as either IIB or IIX by either the myosin ATPase method or by identification of MHC isoforms as IIX.

Single fibres may at times express MHC proteins from a range of classifications. Consequently the term 'hybrid muscle fibres' was coined to describe these fibres (Hamalainen and Pette 1995). Discussions of hybrid fibres are beyond the scope of this review.

2.2.6 Comparison of the Methods used to Classify Muscle Fibres

The terms that have been developed to differentiate between muscle fibre types are often used interchangeably. However, this is an improper use of these terms because each set of definitions was coined to categorise fibres according to the parameter being investigated and fibres defined by one method do not necessarily fit into the same category when grouped according to other criteria. Nevertheless there is considerable overlap between the methods of classification as summarised in Table 2.2.

Table 2.2: Functional and structural characteristics of the three types of skeletal muscle fibres (adapted from Close (1972)).

Characteristic	Type of Fibre		
	Slow Oxidative (Type I)	Fast Oxidative (Type IIA)	Fast Glycolytic (Type IIX)
Myosin ATPase activity	Low	High	High
Speed of Contraction	Slow	Fast	Fast
Force of Contraction	Low	Moderate	High
Fatigue Resistance	High	Moderate	Low
Oxidative Capacity	High	High	Low
Mitochondrial Density	High	High	Low
Myoglobin Content	High	High	Low
Anaerobic Enzyme Content	Low	Moderate	High
Capillary Density	High	High	Low
Glycogen	Low	Moderate	High
Fibre Diameter	Small	Moderate	Large

2.3 SKELETAL MUSCLE METABOLISM

In skeletal muscle cells ATP hydrolysis provides the energy required for all the functions of the muscle, including that required for mechanical contraction. The hydrolysis of ATP to ADP and P*i* and the related release of energy, serves to energise the myosin cross-bridges, converting myosin from a low energy (LE) to a high energy (HE) state. In their HE state, the cross-bridges pull the actin molecules over the myosin molecules shortening the sarcomere and consequently the muscle (Rayment et al. 1993). This reaction is described below.

$$\text{ATP} + \text{Myosin (LE)} \xrightarrow{\text{Myosin ATPase}} \text{ADP} + P_i + H^+ + \text{Myosin (HE)} \qquad (1).$$

However ATP is found in limited quantities in the skeletal muscle with concentrations of ~24 mmol/kg/dry weight (d.w.) of muscle (Hultman 1967) which in the absence of ATP

resynthesis, would be exhausted within 2-3 seconds of intense exercise. As a result, the metabolic pathways responsible for ATP resynthesis are vital for continued muscle contraction. The metabolic pathways can be divided into two groups, the anaerobic pathways (those not requiring oxygen) and the aerobic pathways that take place in the mitochondria.

2.3.1 Anaerobic Pathways of ATP Resynthesis

Exercising muscle uses a combination of all metabolic pathways to resynthesise ATP. The degree to which each pathway is recruited is dependent on the intensity of the exercise. Intense exercise of short duration relies primarily on anaerobic mechanisms to resynthesise ATP. The metabolic pathways involved in this process are described below.

2.3.1.1 Creatine Kinase Reaction

The simplest and most direct method of ATP resynthesis involves the breakdown of phosphocreatine (PCr) into creatine with the energy released being used to resynthesise ATP. PCr is a high energy phosphorylated compound and exists in concentrations three to four times greater in resting muscle than those of ATP (Harris et al. 1974) providing a reservoir of high energy phosphates that are used to regenerate ATP. This reaction is catalysed by the enzyme creatine kinase (equation 2).

$$PCr + ADP \xrightarrow{\text{Creatine Kinase}} Cr + ATP \qquad (2).$$

2.3.1.2 Adenylate Kinase Reaction

During intense exercise, the rate of ATP hydrolysis may exceed the rate of its resynthesis (Stathis et al. 1994). Increases in the concentration of ADP will lead to the activation of the adenylate kinase reaction. This reaction is catalysed by the enzyme adenylate kinase and

involves the hydrolysis of one molecule of ADP to adenosine monophosphate (AMP) to provide the energy for the resynthesis of another molecule of ADP to ATP (equation 3).

$$ADP + ADP \xrightarrow{\text{Adenylate Kinase}} ATP + AMP \qquad (3).$$

This reaction is inhibited by its product, AMP, and in order for this reaction to continue, the increased concentration of AMP must be limited. An increase in the concentration of AMP and the decrease in the ATP/ADP ratio result in the activation of AMP deaminase (Newsholme and Start 1973) which converts AMP to inosine monophosphate (IMP) and ammonia (NH_3) (Jansson et al. 1987)(equation 4).

$$AMP + H^+ \xrightarrow{\text{AMP deaminase}} IMP + NH_4^+ \qquad (4).$$

Despite the capacity of creatine kinase and adenylate kinase for ATP resynthesis there are insufficient energy stores in muscle to sustain exercise for more than 10-15 seconds. Consequently, other energy sources are required for the continued resynthesis of ATP.

2.3.1.3 Glycolysis

Glycolysis involves a series of reactions in which glucose is metabolised to pyruvate with the net production of 2 or 3 ATP molecules, depending on whether the glucose that is used in this process is taken up from the bloodstream (2 ATP produced) or is obtained through the process of glycogenolysis (3 ATP produced). The reactions of glycolysis are summarised in Figure 2.1.

In anaerobic metabolism, pyruvate is converted to lactate by lactate dehydrogenase. Lactate accumulates in the skeletal muscle and is also released into the interstitium and blood stream during exercise (Karlsson and Saltin 1970). An increase in lactate production is indicative of an elevated rate of anaerobic glycolysis (Wasserman et al. 1973) and this can also be influenced by other factors, such as elevations in plasma concentrations of adrenaline (Greenhaff et al. 1991). The activity of the enzyme lactate dehydrogenase (Essen-Gustavsson and Henriksson 1984) and consequently lactate production during exercise (Ball-Burnett et al. 1991) are also much higher in type II muscle fibres than type I muscle fibres meaning that the proportions of different muscle fibre types within a muscle will play a role in the lactate production by that muscle.

The coenzyme nicotinamide adenine dinucleotide (NAD^+), which acts as a hydrogen transporter in metabolism, is also involved in glycolysis. Briefly, NAD^+ is reduced to NADH + H^+ during the glyceraldehyde-3-phosphate dehydrogenase mediated reaction (see Figure 2.1). In aerobic metabolism, the NADH + H^+ transports the hydrogens to the electron transport chain in the mitochondria. In anaerobic metabolism, the NADH + H^+ is oxidised to NAD^+ in the conversion of pyruvate to lactate (see figure 2.1). The NAD^+ that remains following this reaction will again be reduced to NADH + H^+ during subsequent cycles of glycolysis.

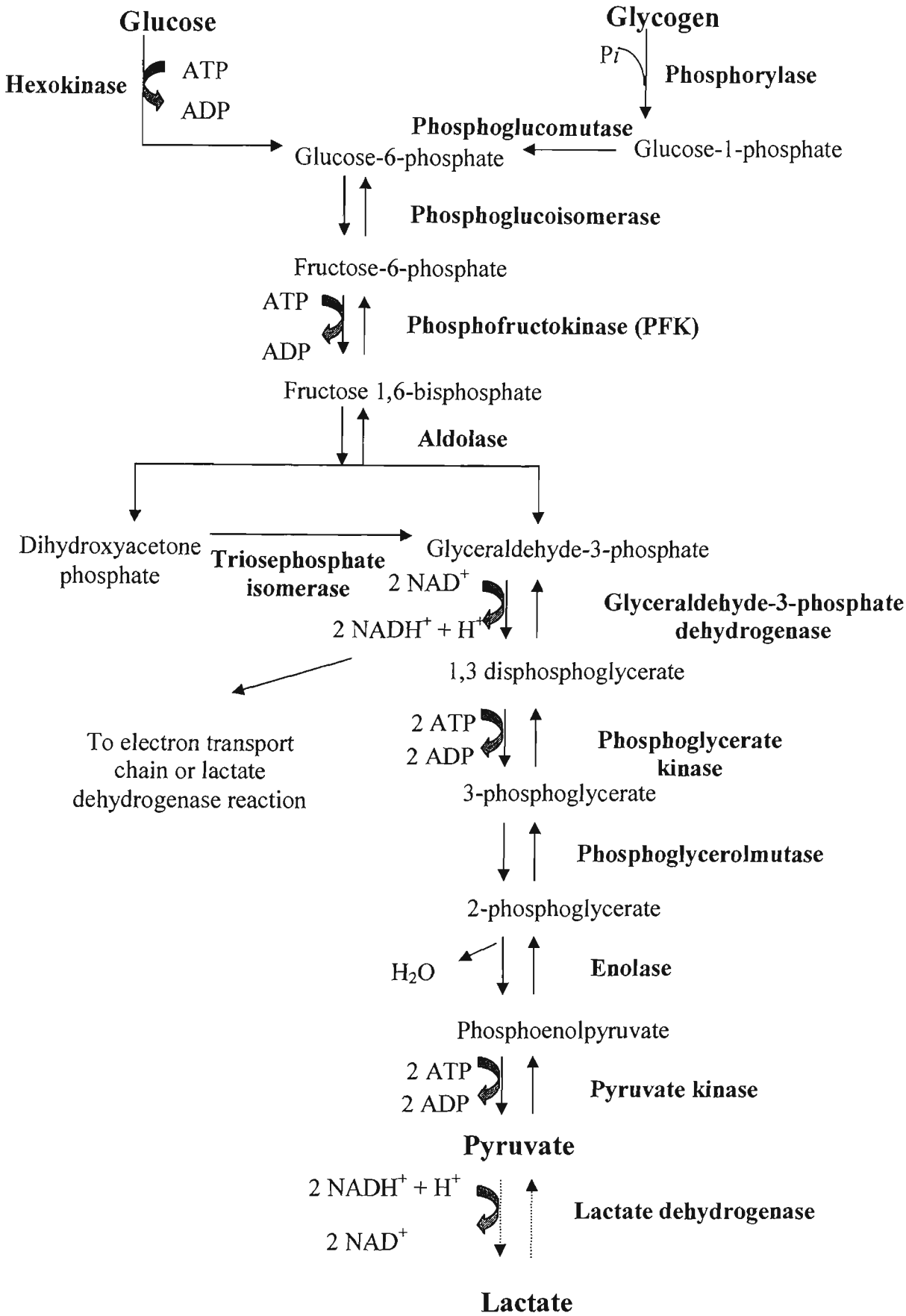


Figure 2.1 Pathway of anaerobic glycogenolysis and glycolysis.

2.3.2 Aerobic Pathways of ATP Resynthesis

Aerobic metabolism occurs in the mitochondria and it has been suggested that it is regulated by intracellular ADP and P*i* concentrations (Stanley and Connett 1991). The process of aerobic metabolism utilises energy derived from the oxidation of carbohydrates, fats and amino acids to resynthesise ATP and involves two cooperating pathways, the Krebs cycle and the electron transport chain. In the Krebs cycle, the oxidation of the fuel sources is completed with the coenzymes NAD^+ and Flavin Adenine Nucleotide (FAD) being reduced to NADH + H^+ and $FADH_2$ and serving as hydrogen transport molecules. In the electron transport chain, H^+ and e^- obtained from the hydrogen carrier molecules are used to resynthesise ATP. Oxidative phosphorylation is the most productive of all the metabolic pathways, providing almost 100% of ATP resynthesis at rest and upwards of 90% during prolonged exercise (Åstrand and Rodahl 1977). At rest and during long duration exercise, free fatty acid (FFA) oxidation is a major source of energy for ATP resynthesis. During the initial stages of long duration exercise and shorter duration exercise of a higher intensity, carbohydrates are preferentially used for metabolism due to a greater ability of the metabolic pathways to oxidise carbohydrate stores at a sufficient rate for ATP resynthesis rate to be maintained via aerobic mechanisms. The contribution of amino acid oxidation to the resynthesis of ATP is generally minimal, but may become significant during the latter stages of long duration exercise when muscle glycogen and blood glucose levels are low (Wagenmakers 1999).

2.3.2.1 Structure of Mitochondria

Mitochondria are the intracellular organelles in which oxidation of substrates take place. These substrates include pyruvate and lactate (the products of glycolysis) as well as the

products of lipid and amino acid metabolism. It is within the mitochondria that almost all oxygen is consumed and ATP rephosphorylated from ADP.

Mitochondria are commonly observed as oval shaped organelles (Palade 1953) and have been categorized as either subsarcolemmal or intermyofibrillar, depending on their location within muscle cells (Hoppeler et al. 1973; Krieger et al. 1980). However it has also been suggested that mitochondria exist as an elaborate network or reticulum within the skeletal muscle rather than as individual ovoid organelles (Kirkwood et al. 1986).

Dual membranes, identified via electron microscopy, enclose the mitochondria (Palade 1953). The outer mitochondrial membrane is 6-7nm thick and contains proteins which form special pores in the membrane (Tyler 1995) and allow many low weight molecules (< 5000 Dalton) to pass easily through the membrane (Nelson and Cox 2000). In addition to these pores, the outer membrane contains a number of enzymes involved in oxidation-reduction (REDOX) reactions and in lipid metabolism (Tyler 1995). The space between the membranes is the intermembranous space and contains some metabolic enzymes, such as adenylate kinase (Ernster and Kuylenstierna 1970). Similar to the outer membrane, the inner membrane is also about 6nm thick and has a large surface area due to a large number of folds or cristae. Generally, the inner membrane is impermeable to most water-soluble molecules and ions, including protons (H^+), but O_2, H_2O, CO_2 NH_3 and some lipid soluble molecules can diffuse directly across the membrane (Sherratt et al. 1988). The inner membrane contains the components of the electron transport chain which include complexes I, II, III, IV and ATP synthase (Hatefi 1985). A number of carriers for the transport of ions, substrates and nucleotides, such as ADP-ATP translocase, are also contained within the inner membrane (Tyler 1995). The inner compartment of the mitochondrion is the matrix.

Enzymes of the Krebs cycle are contained within the mitochondrial matrix and because of the folding of the inner mitochondrial membrane and the respiratory chains contained within this membrane, the distance from any Krebs cycle enzyme molecule to the nearest respiratory chain unit is no more than 10-20nm (Weibel 1984)(pp 110). Additionally, the enzyme succinate dehydrogenase (SDH) is bound to the inner mitochondrial membrane ensuring a tight link between this enzyme and the electron acceptor for this step (FAD) which appears as complex II of the respiratory chain.

2.3.2.2 Krebs Cycle

The oxidation of pyruvate occurs in the cristae of the mitochondria between the folds of the inner mitochondrial membrane. In order to enter the Krebs cycle, the pyruvate produced in glycolysis must first pass through both the outer and inner mitochondrial membranes. In the mitochondrial matrix, the pyruvate is converted to acetyl coenzyme A via the pyruvate dehydrogenase complex resulting in the formation of CO_2 and the reduction of a further molecule of NAD^+ to NADH + H^+. Subsequently the acetate moiety of the acetyl coenzyme A combines with oxaloacetate to form citrate via the citrate synthase reaction which initiates the reactions of the Krebs cycle. In the reactions of the pyruvate dehydrogenase complex (figure 2.2) and the Krebs cycle (figure 2.3), pyruvate is completely oxidised with the consequent formation of 1 molecule of ATP and 4 NADH + H^+ and 1 $FADH_2$ carrier molecules. The $FADH_2$ and NADH + H^+ transport hydrogen to the electron transport chain which is situated along the internal surface of the inner mitochondrial membrane.

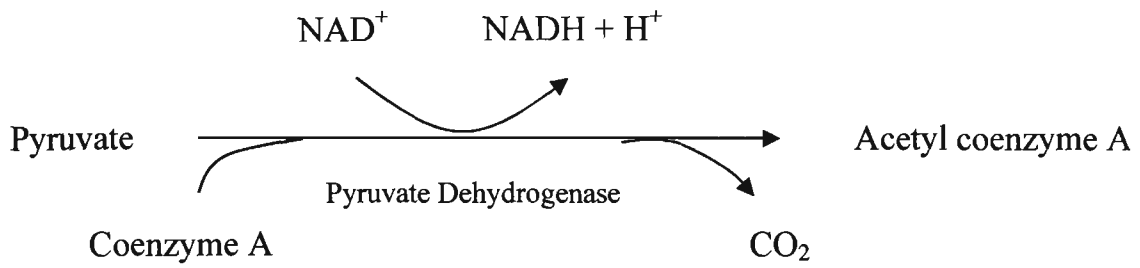


Figure 2.2. Reaction catalysed by the pyruvate dehydrogenase complex.

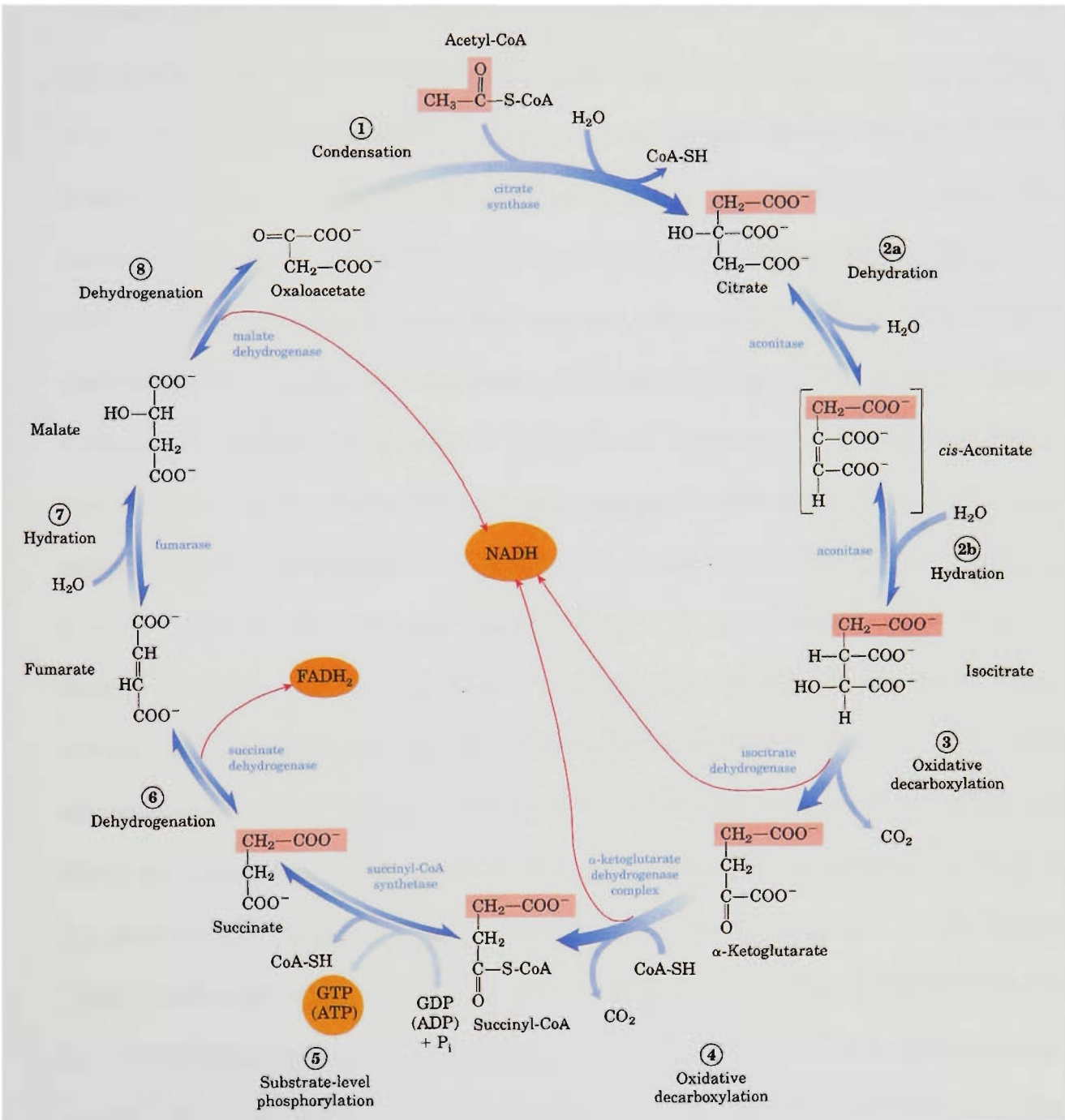


Figure 2.3. Summary of the Krebs Cycle. (Reproduced from (Nelson and Cox 2000) Fig. 16-7. pg 572).

2.3.2.3 The Electron Transport Chain

The electron transport chain is located along the inner surface of the inner mitochondrial membrane. It consists of four respiratory chain components, known as Complexes I, II, III and IV, and ATP synthase, a complex directly involved in ATP formation (Hatefi 1985). The oxidative phosphorylation that occurs in the electron transport chain consists of two separate but linked processes (chemiosmotic theory) that were first described by Peter Mitchell (Mitchell 1965). Complex I, or NADH dehydrogenase receives electrons from NADH + H^+ and transfers these electrons to ubiquinone (UQ) to form reduced ubiquinone (UQH_2) (Weiss et al. 1991) while the associated hydrogen ions are pumped against their concentration gradient through the inner mitochondrial membrane to the intermembrane space. The reaction of complex I can be inhibited by rotenone, a site I inhibitor (Vanden Hoek et al. 1997). Complex II contains FAD and succinate dehydrogenase (SDH). This complex catalyses electron transfer from succinate to FAD and subsequently to UQ (Hatefi 1985). Complex III initiates the transfer of electrons to cytochrome *c* (Trumpower 1990). Cytochrome *c* is located in the intermembrane space and its main function is to continue the transfer of electrons to complex IV. Complex IV directs the electron flow from cytochrome *c* to O_2 to form H_2O (Babcock and Wikstrom 1992). For each NADH + H^+ entering the electron transport chain a total of six hydrogen ions are pumped across the inner mitochondrial membrane while for each FADH entering the electron transport chain, a total of four hydrogen ions are pumped across the inner mitochondrial membrane. The energy that allows the protons to be pumped through the inner membrane comes from the shuttling of the electrons from transport proteins (electronegative redox potential) to atomic oxygen (electropositive redox potential). A region of decreased pH and positive charge is created in the intermembranous space by this movement of the hydrogen ions. The electrochemical gradient for protons that is created by this movement of hydrogen ions ultimately supplies

the energy to resynthesise ATP. Pairs of hydrogen ions re-enter the mitochondrial matrix through ATP synthase, which is arranged along the inner mitochondria membrane, resulting in the resynthesis of ATP from ADP and P*i*. This reaction is shown in equation 5.

$$ADP^{3-} + H_2PO_4^- + 3H^+_{out} \longrightarrow ATP^{4-} + H_2O + 3H^+_{in} \qquad (5).$$

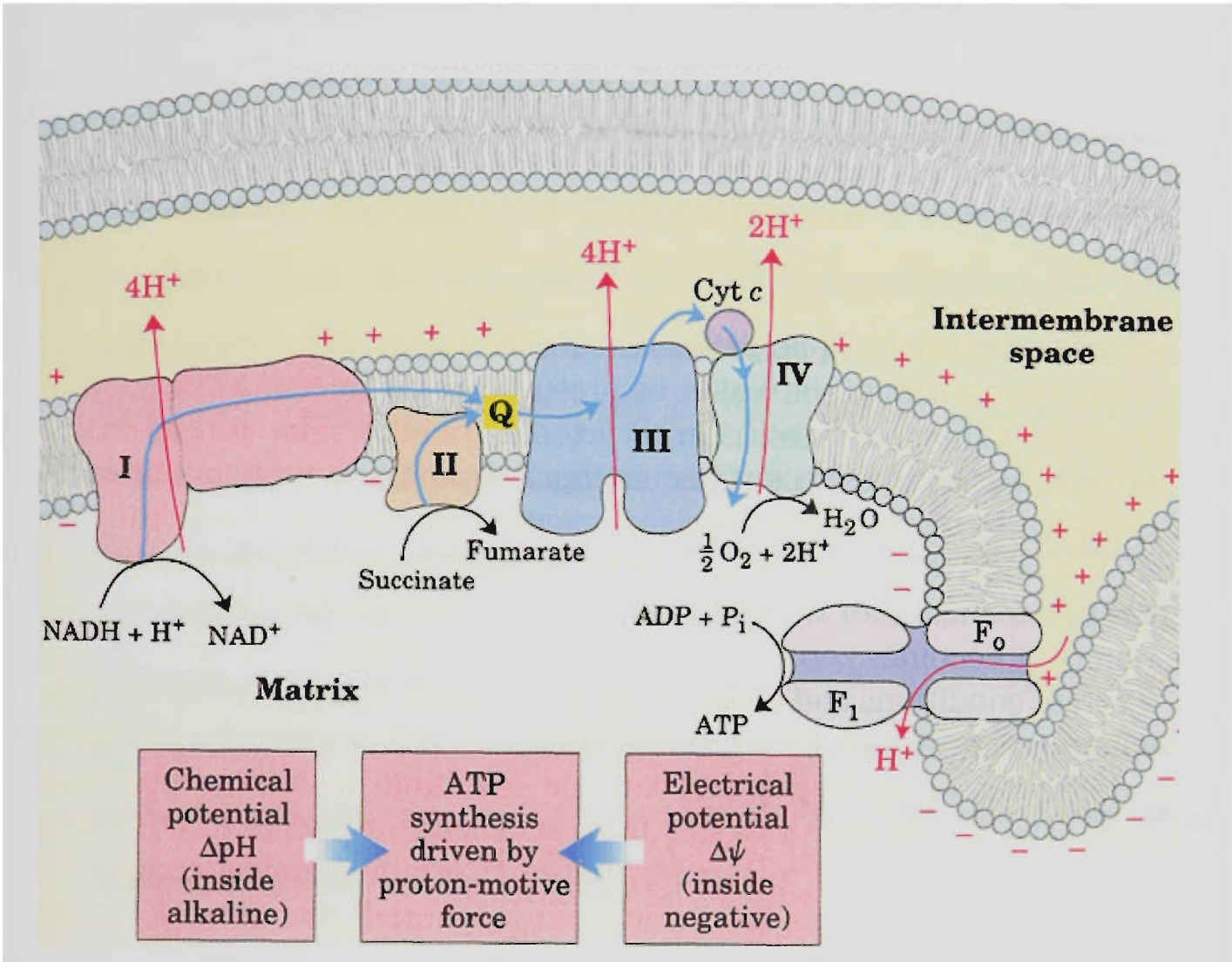


Figure 2.4. Summary of the flow of electrons and protons through the four complexes of the respiratory chain. (Reproduced from (Nelson and Cox 2000) Fig. 19-16. pg 675)

2.3.2.4 Oxidation of Fatty Acids.

The two main processes involved in the oxidation of fatty acids are fatty acid activation and β-oxidation (Schulz 1991). The activation process involves a reaction between the fatty acid, ATP and coenzyme A to form acyl-coenzyme A and AMP (equation 6).

$$\text{Fatty acid} + \text{ATP} + \text{CoA} \longleftrightarrow \text{Acyl-CoA} + \text{AMP} + \text{PP}i \qquad (6)$$

Fatty acid oxidation is referred to as β-oxidation because it involves the sequential removal by oxidation, of 2-carbon units from the β-carbon position of the fatty acyl-CoA molecule. β-oxidation comprises four reaction steps ((Schulz 1991); Figure 2.6) and each cycle of β-oxidation results in the length of the fatty acid chain being reduced by 2 carbon atoms, the formation of an acetyl CoA molecule and the reduction of single molecules of NAD and FAD to NADH + H^+ and $FADH_2$ respectively (Figure 2.6). β-oxidation of a single long chain fatty acid produces more acetyl-CoA, NADH + H^+ and $FADH_2$ than the oxidation of a molecule of glucose. For example, the complete oxidation of a single molecule of palmitate (C_{16}) results in the production of 8 acetyl-CoA, 7 NADH + H^+ and 7 $FADH_2$ resulting in the nett formation of 129 molecules of ATP compared to between 36-38 ATP molecules produced by the oxidation of a single molecule of glucose. Clearly, fatty acid oxidation can be an important producer of ATP for muscle function.

2.3.2.5. Amino acid oxidation

Amino acids can be converted to acetyl-CoA, succinyl-CoA, oxaloacetate, fumarate or α-ketoglutarate (α-KG) depending on the particular amino acid (Figure 2.7a). These products become substrates of the Krebs cycle reactions (Felig 1975). A major route for the conversion of amino acids into oxyacids and ammonia involves the coupled action of the

aminotransferase and glutamate dehydrogenase (GDH) pathways (Goodman and Lowenstein 1977). Many of the amino transferase reactions occur in the cytosol; however GDH is located in the mitochondrial matrix (Figure 2.7b). For example, the amino acid glutamine can be converted by aminotransferase to glutamate in the cytosol. The glutamate is then transported by the glutamate carrier (Glu carrier), which is located in the inner mitochondrial membrane, into the matrix where it is converted by GDH into α-KG, ammonia and NADH + H^+. The α-KG, produced in this reaction can be transported by the α-KG carrier to the cytosol to continue this process (Figure 2.7b) or used as an intermediate in the Krebs cycle (Figure 2.7a).

2.3.3. Summary of metabolism

Intracellular storage of ATP is limited in skeletal muscle and is such that without ATP resynthesis it would be exhausted within 2-3 seconds of exercise commencing. To maintain muscle contraction, the rapid resynthesis of ATP is crucial. Pathways by which ATP is resynthesised include the creatine kinase pathway, the adenylate kinase (myokinase) pathway, glycolysis and aerobic metabolism. The aerobic pathway is sufficient to meet the metabolic demands of resting metabolism. At the onset of exercise, the demand for ATP resynthesis is increased and the anaerobic pathways initially provide for the majority of this resynthesis. Anaerobic pathways also provide for a significant proportion of ATP resynthesis in intense exercise. Aerobic metabolism occurs in the mitochondria, and in exercise bouts lasting for longer than one minute most of the energy is provided via aerobic metabolism.

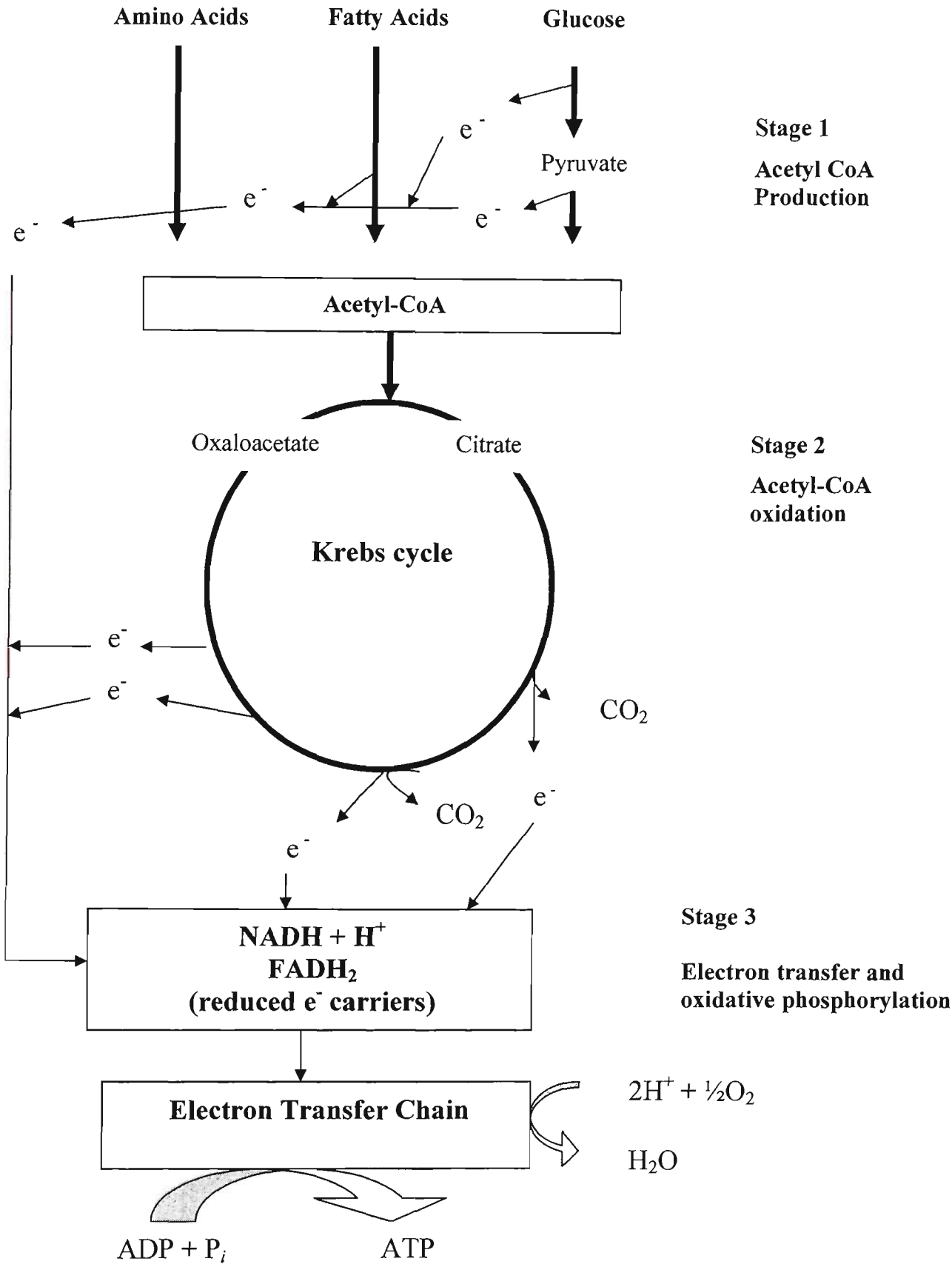


Figure 2.5. Catabolism of Proteins, Fats and Carbohydrates in Cellular Respiration Diagram is redrawn from (Lehninger et al. 1993), p. 447

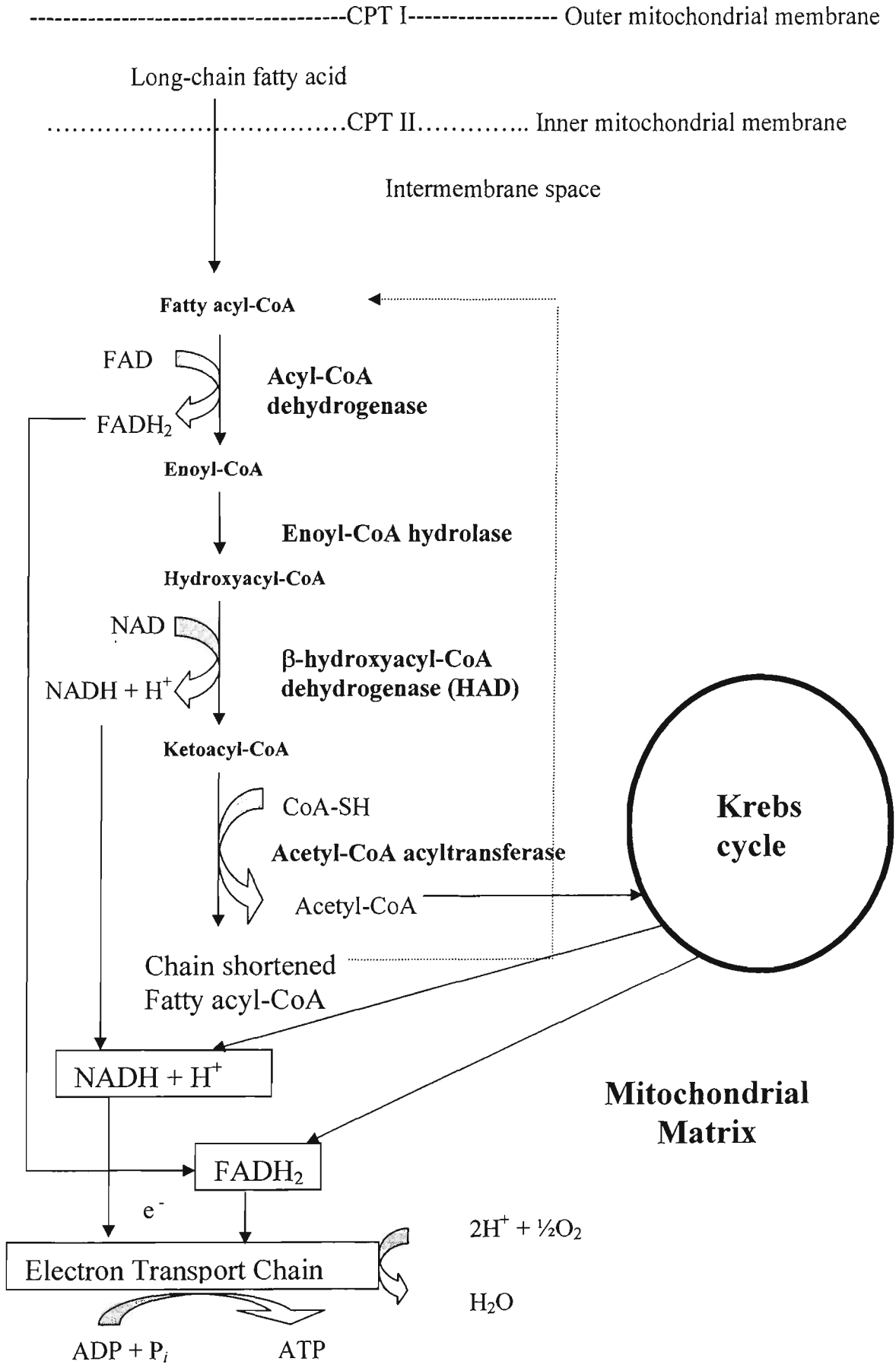
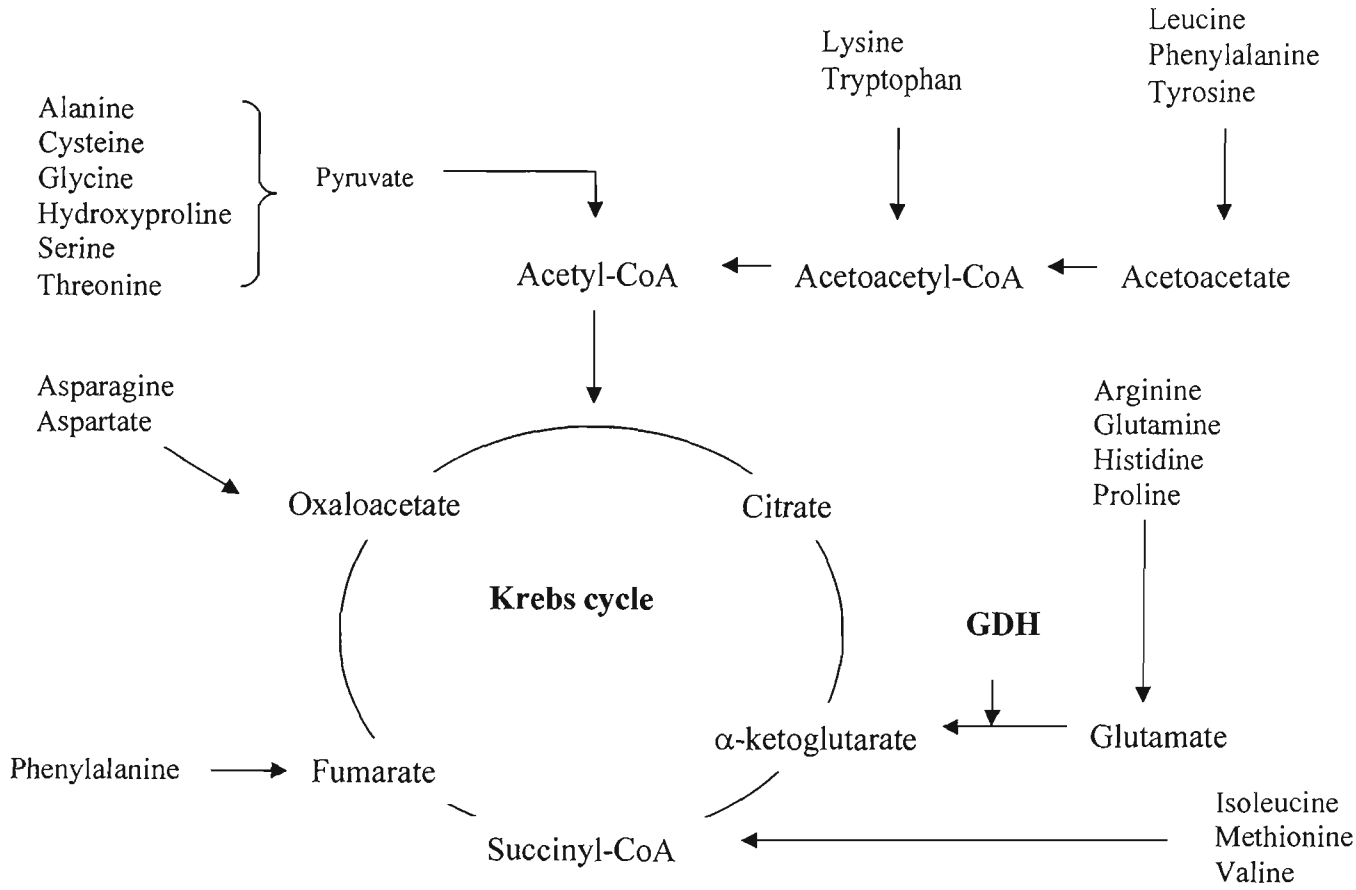
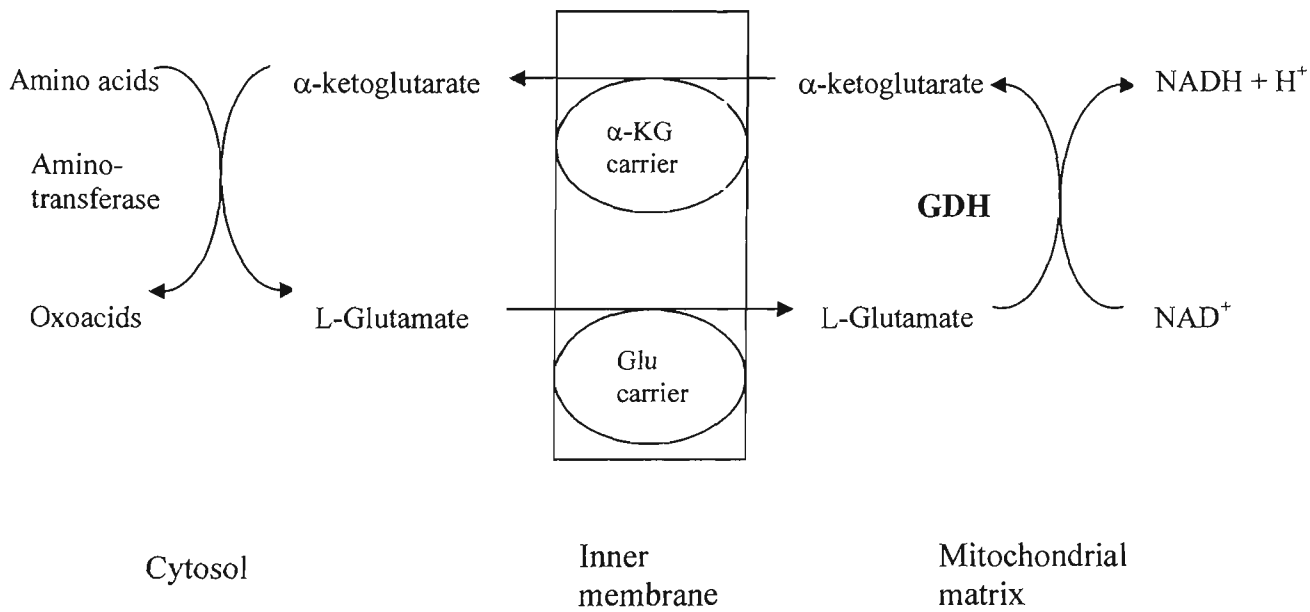


Figure 2.6 Fatty acid activation and β-oxidation. Diagram adapted from (Wolfe 1993), p.360 CPT, carnitine palmitoyl transferase.



(a) Sites of amino acid entry to metabolic pathways



(b) Aminotransferase and GDH reactions

Figure 2.7. Entry of Amino Acids into Metabolic Pathways. Diagrams adapted from (Tyler 1995), pp 82 & 84. GDH, Glutamate dehydrogenase.

2.4 ASSESSMENT OF MITOCHONDRIAL FUNCTION

A range of methods have been used to assess mitochondrial function. Methods used include the determination of mitochondrial enzyme activity and the determination of either mitochondrial oxygen consumption or mitochondrial ATP production *in vitro*. The basic mechanisms of the different methods are described below.

2.4.1 Oxidative Enzyme Activity

Traditionally, analysis of mitochondrial function has relied on the measurement of the activity of oxidative enzymes from homogenised muscle samples (Costill et al. 1976; Chi et al. 1983; Green et al. 1984) such as CS, HAD, SDH or oxoglutarate dehydrogenase (OGDH), or from histochemical stains designed to identify the activity of the mitochondrial enzymes SDH (Andersen and Hoyer 1973) or cytochrome-c-oxidase (COX) (Seligman et al. 1968). These methods rely on the assumption that the enzymes whose activities are measured are present only in the mitochondria. When using homogenised muscle samples the tissue sample is homogenised to disrupt the mitochondria and allow the enzyme that is to be measured to be released into the homogenate. Aliquots of the homogenate are then added to solutions which contain substrates that are involved in the reaction that the enzyme of interest catalyses. The activity of the enzyme can then be determined by measuring either spectrophotometrically or fluorometrically the rate of appearance of a product or disappearence of a substrate (usually a reduced coenzyme such as NADH). Enzyme activity can be quoted either as the activity per unit muscle weight or per unit of muscle protein. The use of enzyme activities as an index of muscle mitochondrial function *in vivo* is, at best, an indirect indicator of muscle mitochondrial function. These determinations are performed under *in vitro* experimental conditions which are not necessarily obtained *in vivo*. Nevertheless, changes in mitochondrial enzyme activities correlate well with other indices of

muscle oxidative capacity such as $\dot{V}O_{2\,max}$ (Carter et al. 2001). In addition, it is well known that endurance training, which increases muscle oxidative capacity, also results in an increase in several of the key enzymes of aerobic metabolism (Klausen et al. 1981; Schantz et al. 1983).

2.4.2 Measurement of Mitochondrial Function With Intact Mitochondria

The complete oxidation of pyruvate or fatty acids to CO_2 and H_2O with the concomitant synthesis of ATP is a complex process. While measurement of the activities of the rate limiting enzymes from biochemical pathways, such as the Krebs cycle, give an indication of maximal rates of flux through these pathways, they can only ever be used to predict the actual rates of flux in an intact system. Rates of ATP resynthesis in intact mitochondria can be more accurately determined by measuring the utilisation of oxygen or by measurement of the products formed (ATP). Three methods of assessing the functional state of the mitochondria are presented below.

2.4.2.1 Polarographic Determination of Mitochondrial Oxygen Uptake

Polarographic methods of measuring oxidative capacity are a commonly employed technique for determining the rates of change of the concentration of oxygen in a solution. This method utilises oxygen to sythesise ATP from ADP and P*i* in isolated mitochondrial preparations. Substrates, such as pyruvate, malate, glutamate and succinate, are commonly used to stimulate oxidative phosphorylation in these experiments.

There are five states of mitochondrial respiration that have been identified previously (Chance and Williams 1955). State 1 is the initial change in the absorbance of the solution. State 2 is the respiration rate of the solution following the addition of ADP to state 1, where

the increase in respiration over that recorded in state 1 is likely to be due to the adenylate kinase reaction and endogenous levels of substrate. State 3 follows the oxidative phosphorylation of ADP with the addition of a substrate such as β-hydroxy butyrate. State 4 corresponds to the exhaustion of ADP and state 5 occurs when the mitochondrial suspension becomes anaerobic. Mitochondrial ATP production by oxidative phosphorylation occurs in state 3.

The mitochondrial respiratory chain utilises oxygen to synthesise ATP from ADP and P*i*. The most common procedure to estimate P/O ratios is from the O_2 uptake recorded polarographically following the addition of a known amount of ADP. The number of moles of ADP or P*i* consumed in this process is equal to the number of moles of ATP synthesised. In theory oxidation of one mole of NADH + H^+ should give a P/O ratio of 3. (equation 7).

$$NADH + H^+ + \tfrac{1}{2} O_2 + 3ADP + 3Pi \longleftrightarrow NAD^+ + H_2O + 3ATP. \qquad (7)$$

In contrast, the oxidation of one mole of $FADH_2$ should give a P/O of 2. (equation 8)

$$FADH_2 + \tfrac{1}{2} O_2 + 2ADP + 2Pi \longleftrightarrow FAD + H_2O + 2ATP \qquad (8)$$

In practice however, these P/O ratios do not occur, as electron transport is not completely coupled to ATP synthesis. Some protons in the mitochondrial intermembrane space leak down their concentration gradient back across the inner membrane directly, rather than through the ATP synthase pathway. This proton leak is considerable and has been reported in many tissues including skeletal muscle (Rolfe and Brand 1996). Indeed, proton leak across the inner mitochondrial membrane has been reported to account for 52% of the oxygen

consumption of resting perfused rat muscle (Brand et al. 1994) and the proton cycle in working muscle and liver has been estimated to account for 15% of standard metabolic rate in rats (Rolfe et al. 1999). Consequently, linking oxygen consumption to ATP production rate is fraught with potential error and a better method of determining oxidative capacity *in vitro* may be to measure the rate of ATP production under optimal conditions.

2.4.2.2 Mitochondrial ATP Production Rate

Mitochondrial ATP production rate (MAPR) is a method of determining muscle oxidative capacity that is performed using mitochondria isolated from biopsy samples. The assay relies on a luminometric analysis using firefly luciferase in which light that is emitted is proportional to the ATP concentration over the range of 10^{-11} to 10^{-6} mol.L^{-1} (Lundin et al. 1976). Consistent with the measurement of mitochondrial O_2 consumption, MAPR requires the presence of ADP, P*i* and oxidative substrate. A range of substrates can be chosen for this assay, with the choice of substrates reflecting maximal flux through particular oxidative pathways. For example, substrates which are intermediates of carbohydrate oxidation, such as pyruvate and malate, can be used to determine the maximal rate of flux through the Krebs cycle and complex I in the electron transport chain. Similarly, intermediates of amino acid or fat oxidation, such as α-ketoglutarate or palmitoyl-carnitine can be used to simulate protein or fat metabolism. Succinate and rotenone can be used together to determine mitochondrial complex II function. Rotenone, an electron transport chain inhibitor is used to block electron transfer from complex I to UQ, such that electron flux occurs only from complex II down. When using these substrates, electrons are produced through the succinate dehydrogenase mediated reaction. Finally, the combination of pyruvate, malate, palmitoyl-carnitine and α-ketoglutarate can be used to measure the combined capacity for carbohydrate, fatty acid and amino acid metabolism (Wibom et al. 1992).

The rate of ATP production in this assay is significantly correlated with maximal mitochondrial oxygen consumption as determined via the polarographic technique (Tonkonogi and Sahlin 1997). Unlike the polarographic method which requires 1-2 gm of tissue, MAPR requires a much smaller sample (30-40mg) of tissue (Wibom et al. 1990) meaning that a needle biopsy sample is sufficient to harvest the tissue required for this assay. Previously, MAPR has been demonstrated to provide a comprehensive assessment of muscle oxidative capacity through the entire range of metabolic pathways from which mitochondria generate ATP (Wibom et al. 1990). MAPR is higher in endurance trained individuals compared to sedentary subjects (Wibom and Hultman 1990), increases with endurance training (Wibom et al. 1992; Starritt et al. 1999) and decreases following a detraining period (Wibom et al. 1992). Additionally in lung transplant recipients $\dot{V}O_{2\ peak}$ is also much reduced which is likely to be due at least in part to the significantly reduced MAPR found in this group (Wang et al. 1999). A common disadvantage of both the MAPR and the polarographic methods of determining mitochondrial respiration is that both methods are performed on isolated tissue samples *in vitro*. Previously, Tonkonogi and Sahlin (1997) have compared MAPR and the rate of production of ATP predicted from mitochondrial oxygen consumption. They reported that MAPR in the presence of the substrates pyruvate and malate is much lower than ATP production calculated from mitochondrial oxygen consumption while the values were little different when the substrate α-ketoglutarate was used. It is possible that MAPR may not totally reflect maximal oxygen consumption. Nevertheless, it is still a valid and comprehensive measure of muscle oxidative capacity and has been used widely (Wibom and Hultman 1990; Wibom et al. 1990; Bárány et al. 1991; Wibom et al. 1992; Berthon et al. 1995; Starritt et al. 1999; Wang et al. 1999; Hou et al. 2002).

2.4.2.3 Nuclear Magnetic Resonance (NMR) Spectroscopy

A recent method of investigating the rate of oxidative phosphorylation *in vivo* utilises NMR spectroscopy. The nuclei of certain atoms such as hydrogen (^{1}H), carbon (^{13}C) and phosphorus (^{31}P) have net magnetic spin. Phosphorous containing metabolites such as ATP, PCr and P*i* can be observed using NMR spectroscopy because of the net magnetic spin of these atoms. PCr resynthesis post exercise is an oxidative process (Kemp et al. 1993; Paganini et al. 1997). During recovery from exercise, the PCr resynthesis rate is reflective of the rate of mitochondrial ATP synthesis (McCully et al. 1993; Stratton et al. 1994) and has been demonstrated to correlate well with muscle CS activity (McCully et al. 1993).

2.4.3 Role of ADP and Creatine in Oxidative Function

Traditionally, control of ATP production via the process of oxidative phosphorylation was believed to be regulated through feedback of cytosolic ADP and P*i* (Chance and Williams 1955). More recently, it has been suggested that different muscle fibre types respond differently to elevated levels of intracellular ADP. Kay et al. (1997) investigated the role of ADP in stimulating oxidative phosphorylation in rat skeletal muscle and reported a high K_m for ADP in the regulation of oxidative phosphorylation in skinned soleus and cardiac muscle fibres, which are composed primarily of slow twitch (oxidative) fibres. This exceeded the K_m for isolated mitochondrial preparations by a factor of 25. However, this was not the case in the white gastrocnemius muscle which is composed of predominantly type IIB fibres. The authors concluded that in slow twitch muscle cells *in vivo,* there is a low permeability of mitochondrial outer membrane porins for ADP necessitating a carrier molecule of some sort to transport P*i* molecules from the mitochondria for ATP resynthesis in the cytoplasm. It is proposed that ADP still acts to regulate oxidative metabolism, although the supply of ADP

for oxidative phosphorylation appears to be different between oxidative and glycolytic muscle fibres. In glycolytic muscle, ADP diffuses directly across the outer mitochondrial membrane into the mitochondrial matrix via an adenine nucleotide translocase (ANT). ADP also controls respiration in oxidative muscle, although the mechanism is somewhat different. As already stated, the permeability of the outer mitochondrial membrane to adenine nucleotides *in vivo* is low in this type of muscle. The K_m for ADP in oxidative muscle is high but has been demonstrated to decrease dramatically in the presence of elevated levels of creatine (Kuznetsov et al. 1996). As a result, the control of mitochondrial respiration in oxidative muscle is unlikely to be directly due to elevated levels of cytosolic ADP but rather that the 'Phosphocreatine Shuttle' first suggested by Bessman and Savabi (1988) may be involved in cytosolic ATP resynthesis (Figure 2.8).

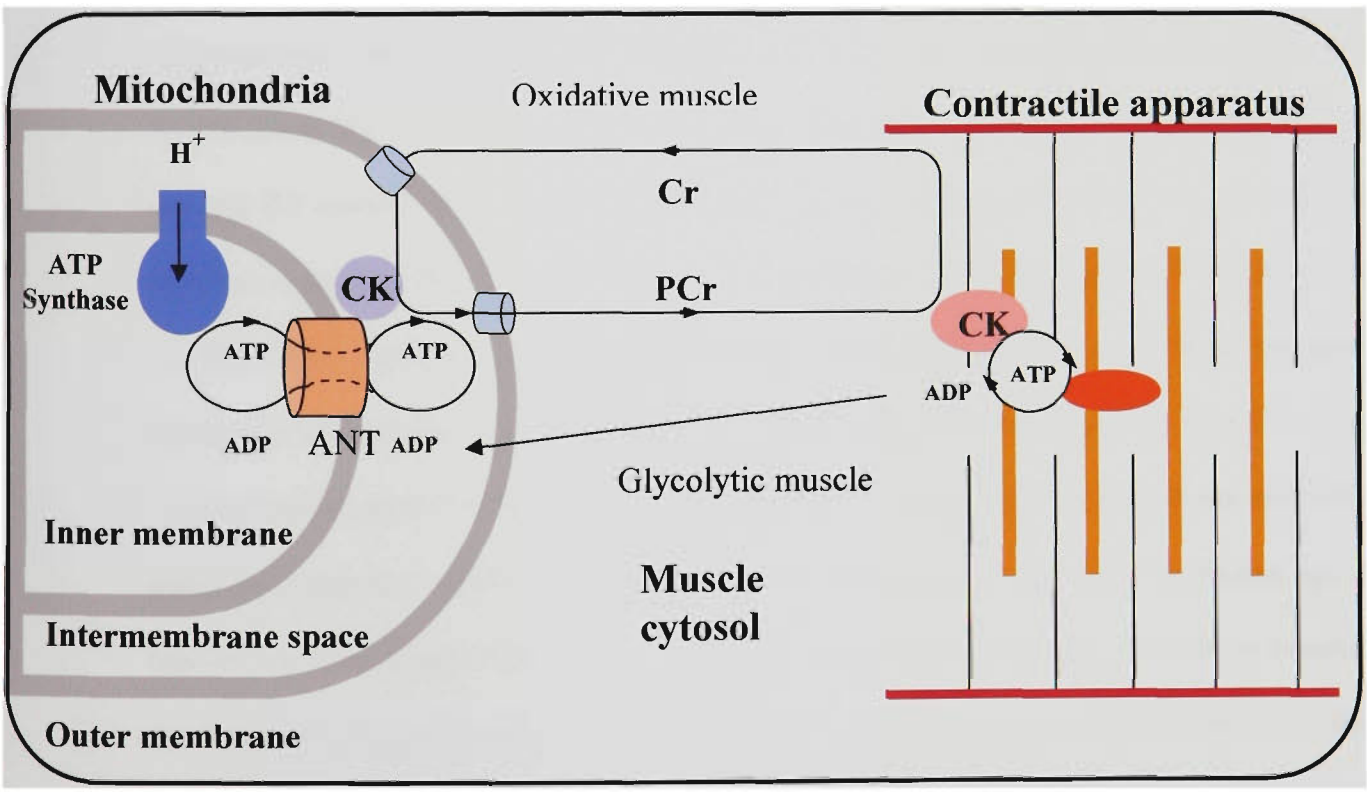


Figure 2.8. The phosphocreatine pathway for intracellular energy transport. Redrawn from (Saks et al. 2000).

2.5 FACTORS AFFECTING MUSCLE STRUCTURE AND METABOLISM IN HEALTHY INDIVIDUALS

Skeletal muscle constitutes approximately 30% of total body mass and is a highly plastic tissue that adapts rapidly to changing functional demands. Many factors have previously been reported to affect the structure or metabolic characteristics of skeletal muscle. Some of these factors include gender, age and physical activity levels. These factors are discussed in more detail below.

2.5.1 Age

The effects of aging on skeletal muscle are many and varied. Previously, decreases in muscle mass, strength and endurance have been described in human subjects with increased age (Coggan et al. 1992b). In healthy young individuals, glycolytic muscle fibres have the largest diameter while oxidative fibres are the smallest (Hughes and Schiaffino 1999). With increasing age, the muscles atrophy (Frontera et al. 2000), and type II muscle fibres decrease in size (Frontera et al. 2000). Cross-sectional studies have reported that in elderly subjects, the type IIX muscle fibres are the smallest with the type I (Coggan et al. 1992a; Proctor et al. 1995) or type IIA fibres (Essén-Gustavsson and Borges 1986) the largest. It has also been suggested that aging results in a shift in muscle fibre proportions towards an increased percentage of oxidative type I muscle fibres at the expense of the glycolytic type IIX fibres (Aoyagi and Shephard 1992). Many investigators have examined this hypothesis and while some have reported a shift in fibre proportions towards slow fibres with increasing age in rats (Kovanen and Suominen 1987) and humans (Frontera et al. 2000), others have reported no change in muscle fibre proportions with aging (Essen-Gustavsson and Henriksson 1984; Coggan et al. 1992a; Proctor et al. 1995). However, it has been suggested that the shift in muscle fibre proportions that have been reported to occur with healthy aging in humans

(Frontera et al. 2000) may not appear until the eighth decade of life and those investigators who have reported no shift in skeletal muscle fibre proportions have tended to use subjects in their 50s and 60s as their aged subject group. Regardless of the effect of aging on proportions of type I and II fibres however, it is clear that there is a decrease in the relative type II fibre contribution to skeletal muscle with aging. This may be the result of disuse atrophy occurring due to a decrease in activity, particularly high intensity activity, with increasing age. Reduced recruitment of the glycolytic fibres and consequently a reduction in their size would be expected to accompany such a fall in intense activity.

Healthy aging has also been noted to affect muscle oxidative capacity. Houmard et al. (1998) reported a negative correlation between age and the activity of the oxidative enzyme CS in gastrocnemius but not vastus lateralis muscle. Additionally, when subjects were divided into those aged less than 30 years and those aged more than 60 years, CS activity from gastrocnemius but not vastus lateralis muscle was reported to be significantly lower in the elderly subjects. In contrast, oxidative capacity, represented by the activity of the enzyme CS, was reduced in the vastus lateralis of elderly subjects examined by Essén-Gustavsson and Borges (1986). However, the activity of the enzyme HAD, the rate limiting enzyme of the beta-oxidation of fatty acids, was reportedly not different between any of the age groups investigated, suggesting that the capacity to oxidise fatty acids is unaltered with aging. Using substrate combinations representing carbohydrate and protein metabolism, Trounce et al. (1989) reported a negative correlation between mitochondrial state 3 respiration and age. Additionally the fractional rate of muscle mitochondrial protein synthesis has been reported to be significantly lower in middle aged (mean 54 years) and elderly (mean 73 years) than young individuals (mean 24 years) (Rooyackers et al. 1996). The effect of age on skeletal

muscle oxidative capacity is unclear and differences may be related to methodology and/or the muscle investigated.

Conley et al. (2000) used NMR spectroscopy to monitor aerobic recovery as indicated by PCr resynthesis, following two minutes of supramaximal electrical stimulation via the femoral nerve of the quadriceps muscle group. They reported a lower rate of PCr recovery in elderly (68.8 ± 5.9 years; mean ± SEM) subjects compared with younger adult (38.8 ± 7.9 years) subjects. Muscle biopsies were also taken in this study to determine the mitochondrial volume density of the muscle. Mitochondrial volume density was lower in the elderly compared to the young group but oxidative capacity remained lower in the elderly when mitochondrial density was taken into account (Conley et al. 2000). In contrast, Kent-Braun and colleague (2000) reported no difference in PCr resynthesis rates in the first 15 seconds of recovery following a 15 second maximal voluntary contraction in the tibialis anterior. Although this study employed exercise of a sufficient intensity to severely reduce PCr stores, it is doubtful whether 15 seconds of recovery is sufficient to make any conclusions about PCr resynthesis. The authors did note that post exercise muscle pH was lower following the maximal voluntary contraction (MVC) in the young versus the elderly subjects. This is likely due to a reduced MVC in the elderly per unit of muscle that may be due to reductions in the proportion of type II muscle fibres in the elderly (Frontera et al. 2000) and decreased glycolysis due to the decreased size of the type II glycolytic muscle fibres (Coggan et al. 1992a; Proctor et al. 1995; Frontera et al. 2000).

2.5.2 Gender

Few studies have investigated the effect of gender on skeletal muscle parameters. Muscle oxidative capacity, as indicated by intermyofibrillar mitochondrial volume density, has been

reported to be approximately 1.5 times higher in males than females (Hoppeler et al. 1973). The activity of the mitochondrial enzyme SDH is also greater in males (Nygaard 1981; Green et al. 1984) although the activity of other mitochondrial enzymes, such as CS (Nygaard 1981; Grimby et al. 1982) and HAD (Grimby et al. 1982; Green et al. 1984; Essén-Gustavsson and Borges 1986), are not different.

Essen-Gustavsson et al. (Essén-Gustavsson and Borges 1986) studied 34 male and 31 female subjects from a wide range of ages (20-70 yrs) and reported a significantly higher percentage of type I muscle fibres in the male subjects. In contrast, women displayed a higher percentage of type IIA fibres. There was no difference between proportions of type IIX fibres between the genders. Muscle fibre areas were also reported to be larger in males than females over the age of 30 years. Muscle oxidative capacity, as indicated by the activity of CS, was significantly higher in men than women in the 30 and 40 years age groups, but lower in men aged 60 years compared to women of the same age. In two of the three glycolytic enzymes measured (PFK and HK), there was no difference between the groups, however, the activity of the enzyme triose phosphate dehydrogenase (TPDH) was lower in the female than the male subjects.

Grimby et al. (1982) investigated 78-81 year old male and female subjects and found elevated proportions of IIX muscle fibres in the vastus lateralis of the women compared to the men. Mean fibre areas were lower in the aged females than the aged male subjects, however, the only fibre type in which this was significant was the type IIA fibres in both the vastus lateralis and biceps brachii muscles. Capillary to fibre ratio was also lower in the aged females than the male subjects, although when fibre size was taken into account and capillary density reported as capillaries per mm^2 there was no difference between the

genders. Male subjects had lower activities of the glycolytic enzymes HK and LDH, suggesting a reduced maximal flux through glycolysis. This is consistent with the observation that females tend to have a higher proportion of the more glycolytic type II muscle fibres than their male counterparts.

2.5.3 Physical Activity

Skeletal muscle adapts to the level of physical activity or stress under which it is placed. Adaptations that occur with various forms of training will disappear over time if the training ceases. Many studies have examined the effects of exercise training; detraining or limb unloading that has been designed to simulate the effects of low gravity environments, on skeletal muscle.

2.5.3.1 Detraining or Immobility

If exercise training results in alterations in skeletal muscle, then it stands to reason that ceasing training or detraining may result in the reversal of these adaptations. It has long been assumed that muscular function would be impaired following prolonged periods in weightless conditions. Data from both rats (Deschenes et al. 2001) and humans (Edgerton et al. 1995) have reported significant muscle atrophy following periods in space of between 4 and 11 days. Studies using immobilisation techniques to simulate the effects of micro gravity have been widely studied with similar findings. Desplanches et al. (1987) reported smaller soleus muscles in rats following five weeks of hind limb immobilisation than were found in the control animals. This atrophy appeared to be due to a decrease in the cross-sectional area of all muscle fibre types. Additionally, the authors noted a decrease in the capillary to fibre ratio in the soleus muscle, although when the capillary density was quoted in capillaries/mm^2 there was no difference between the hind limb immobilised and control animals. The

extensor digitorum longus (EDL) muscle was also reported to be smaller in the experimental group when compared to the control animals after five weeks of immobilisation. However, no other measures were significantly different. The authors believed that the greater differences seen between the groups in the soleus were due to its role as a postural muscle, meaning that it was in constant use in the control animals. Similar findings have been reported in human subjects following periods of limb immobilisation. Ferretti et al. (1997) subjected seven subjects to 42 days of head-down tilt bed rest and reported a decrease in muscle fibre area and total muscle cross-sectional area. However, the fibre type distribution remained unaltered.

Muscle morphology can also be affected in subjects who cease training following chronic involvement in exercise training. Muscle fibre cross-sectional areas decrease towards pre-training values in resistance trained subjects following detraining periods of between 12 and 30 weeks (Houston et al. 1983; Staron et al. 1991; Taaffe and Marcus 1997) with a reversal of training induced adaptations in muscle fibre type proportions only appearing after longer periods of detraining (Staron et al. 1990).

Periods of detraining or immobilisation have been demonstrated to adversely effect muscle oxidative capacity. Mitochondrial oxygen consumption in rats is reduced following only one (Max 1972) or two days (Krieger et al. 1980) of hind limb immobilisation. Yajid et al. (1998) investigated the effect of four weeks of hind limb suspension on mitochondrial respiration and reported a decrease in the state 3 respiration of gastrocnemius muscle using the substrates pyruvate and malate. The reported decrease in mitochondrial respiration was only significantly different in the intermyofibrillar mitochondria where mitochondrial respiration was reduced by 59%, although there was also a non-significant reduction in the

respiration of subsarcolemmal mitochondria of 18%. However, when the authors used the substrates succinate and rotenone, they found no difference in either the intermyofibrillar or subsarcolemmal mitochondria between the control and hind limb suspended animals. These results indicate that the function of complex I is significantly reduced following hind limb suspension with no such effect on complex II. Studies that have investigated alterations in the activity of mitochondrial enzymes have reported decreases in the activity of SDH (Booth 1978; Gardiner et al. 1992), suggesting a possible reduction in the function of complex II, CS (Fell et al. 1985; Desplanches et al. 1987) and cytochrome *c* (Fell et al. 1985) following hind limb immobilisation and hypokinesis.

Immobilisation or detraining studies have also reported alterations in muscle oxidative function in human subjects. Mitochondrial volume density has been reported to decrease by 17% and total mitochondrial volume by 29% following seven weeks of head-down tilt bed rest in a group of healthy young males (Ferretti et al. 1997). Decreases in the activity of SDH, CS and other mitochondrial enzymes have also been reported as a consequence of muscle disuse (Haggmark et al. 1981; Halkjaer-Kristensen and Ingemann-Hansen 1985; Jansson et al. 1988; Ferretti et al. 1997). The cessation of training in well-trained individuals has also been demonstrated to cause a similar effect to muscle oxidative capacity as that reported in immobilisation studies. The activity of the oxidative enzymes CS and SDH are reduced following 12 days of detraining in endurance trained subjects (Coyle et al. 1984) while three weeks of detraining has been reported to result in a significant decrease in human skeletal muscle MAPR across the full range of substrates measured (Wibom et al. 1992).

2.5.3.2. Endurance Training

An endurance training stimulus to skeletal muscle can be defined as one in which there is a large increase in the recruitment frequency of motor units with a modest increase against which the motor units must contract (Brooks et al. 2000) (pp 406). The effects of this form of training on skeletal muscle have been widely studied and the adaptations are well understood. The key results from the major studies discussed in this review are presented in Table 2.3.

Overall muscle oxidative capacity increases following endurance training protocols and this is mainly due to increases in the activity of oxidative enzymes (Klausen et al. 1981; Schantz et al. 1983; Proctor et al. 1995) and increased mitochondrial volume density (Hoppeler et al. 1973; Staron et al. 1984). These increases in mitochondrial volume and enzyme activity indicate that there is an increase in mitochondrial oxidative activity which is supported by the reported increases in mitochondrial oxygen consumption (Davies et al. 1981) and MAPR (Wibom et al. 1992; Berthon et al. 1995; Starritt et al. 1999) following endurance training.

Adaptations in the contractile apparatus as indicated by fibre type proportions and fibre cross-sectional areas are minimal with this form of training, although there is evidence suggesting a shift from glycolytic to oxidative muscle fibres with endurance training (Sullivan et al. 1995; Demirel et al. 1999) in animal models and in human studies (Andersen and Henriksson 1977; Coggan et al. 1992b).

In healthy populations, endurance training increases the capillary density of the musculature (Andersen and Henriksson 1977). Cross-sectional studies comparing endurance trained to untrained subjects have reported capillary to fibre ratio, capillary contacts per fibre and

capillary density (capillaries/mm^2) are all higher in endurance trained subjects suggesting an elevated ability to deliver oxygen to the exercising muscles (Proctor et al. 1995; Rodriguez et al. 2002). Studies that have examined changes in capillary density following endurance training protocols have also reported increases in capillary density following training (Denis et al. 1986; Coggan et al. 1992b; Hepple et al. 1997).

The reported shifts towards more oxidative muscle fibres and elevations in mitochondrial volume combined with an improved potential oxygen delivery are likely to have a major role in the improvements in endurance performance that are seen following endurance training.

Table 2.3. The Effects of Endurance Training on Muscle Fibre Type, Capillary Density and Metabolic Capacity

Source	Age(year) /Sex	$\dot{V}O_{2\,max}$ (%)	Muscle	Training	CSA Changes (%)			MAPR	Metabolic Enzymes				Capillary Density		Mitochondria	
					I	IIA	IIX		CS	SDH	HAD	PFK	Cap/Fibre	Cap/mm^2	VD	V/M
(Andersen and Henriksson 1977)	21/M	↑*	VL	8 wk						↑*			↑*	↑*		
(Klausen et al. 1981)	M	↑15*	VL	8 wk									↑*	↑*		
(Schantz et al. 1983)	M		TB						↑*		↑50*		↑40*			
(Staron et al. 1984)	24/M		VL													↑*
(Denis et al. 1986)	22/M	↑15*	VL	20 wk	↔	↔	↔						↑	↑ 25*		
(Denis et al. 1986)	62/M	↑7*	VL	20 wk	↓*	↓*								↑ 33*		
(Coggan et al. 1992b)	64/M&F	↑23*	Gas	9-12mo.	↑*	↑*			↑*	↑*	↑*	↔		↑ 21*		
(Wibom et al. 1992)	20/M	↑10*	VL	6 wk				↑*	↑43*							
(Berthon et al. 1995)	63/M	↑6*	VL	6 wk				↑*	↑36*		↔					
(Hepple et al. 1997)	65-74/M	↑20*	VL	18 wk		↔							↑30*	↑39*		
(Starritt et al. 1999)	20/M	↑9*	VL	10 d.				↑*	↑9*							
(Jubrias et al. 2001)	69/M&F		VL	24 wk		↔									↔	

Changes = (ET – Controls)/controls x 100. TB, Triceps Brachii; VL, Vastus Lateralis; Gas, Gastrocnemius; VD, Volume Density; V/M, volume/myofibrils ratio; MAPR, mitochondrial ATP production rate; ↑, increased; ↓, decreased; ↔, unchanged; * $p < 0.05$.

2.5.3.3. Resistance Training

Resistance training and strength training are terms used to describe a form of training in which muscles contract against an external resistance. In comparison to adaptations found with endurance training, resistance training results in increases in the frequency of motor unit recruitment and significant increases in the force produced by individual motor units (Brooks et al. 2000). Following the onset of resistance training in previously untrained subjects, the early increases in strength are due to central nervous system adaptations which result in increased motor unit recruitment, increased motor unit firing rates and enhanced motor unit firing patterns (Moritani and deVries 1979). These adaptations precede the changes that occur in the contractile proteins and constitute the major improvements in muscle contractile force in the first few weeks of resistance training (Sale 1988).

Effect of Resistance Training on Fibre Proportions and Cross-Sectional Area.

The major adaptation that occurs with continued heavy resistance training is an increase in the cross-sectional area of the trained muscles (Staron et al. 1984; Tesch et al. 1987; Alway et al. 1988; Staron et al. 1991; Wang et al. 1993; Green et al. 1998). This can occur either as a result of hypertrophy (an increase in muscle fibre diameter) or hyperplasia (an increase in the number of muscle fibres within a muscle). Gonyea (1980) reported an increase in the number of muscle fibres of up to 5% in resistance trained limb muscles in cats. These findings have not been replicated in human subjects however, and it is generally accepted that most of the increase in muscle size due to strength training occurs as a result of hypertrophy (Costill et al. 1979; Gollnick et al. 1981; Staron et al. 1990).

Cross-sectional studies have compared long-term resistance-trained subjects with sedentary controls and reported the resistance-trained subjects to have larger thigh masses than

untrained subjects (Staron et al. 1984). When cross-sectional area of fibres have been investigated, it has been reported that the muscle fibres of the vastus lateralis (Staron et al. 1984) and triceps surae (Alway et al. 1988) are larger than those of untrained control subjects. While larger fibres have been noted across all fibre types, type II fibres of the vastus lateralis muscle were reported to be 33% larger than those in untrained subjects while the type I fibres were only 12% larger (Staron et al. 1984) suggesting a fibre type specific response to resistance training. These results are supported by those reported in shorter duration longitudinal studies involving male and female subjects from a range of age groups. While several investigators have reported no significant increase in thigh mass (Luthi et al. 1986) or fibre cross-sectional area (Costill et al. 1979) following resistance training, the training periods of seven and six weeks, respectively, may not have been sufficiently long to note any significant adaptations in the skeletal muscle (Sale 1988). A further study (Cote et al. 1988), which used a more realistic resistance training period of 10 weeks, also reported no increase in fibre cross-sectional area or thigh mass following the training intervention. However, this study used isokinetic equipment which excludes an eccentric component, effectively halving the training stimulus. An eccentric component to resistance training may be important for hypertrophy to occur as the majority of damage that results in skeletal muscle remodelling and hypertrophy is produced with this type of contraction (Hortobagyi et al. 2000). Regardless of these results, the majority of intervention studies have reported an increase in either thigh mass (Jubrias et al. 2001) or fibre cross-sectional area (Tesch et al. 1987; Frontera et al. 1990; Staron et al. 1991; Wang et al. 1993; Green et al. 1998; Hikida et al. 2000).

Fibre proportions have also been noted to change with resistance training, with a reduction in the percentage of type IIX fibres accompanied by a resultant increase in the percentage of

type IIA fibres (Green et al. 1998; Hikida et al. 2000) or a non significant increase in the proportions of both type I and IIA fibres (Staron et al. 1991). Several studies have investigated the overall fibre type contribution to skeletal muscle following resistance training by calculating the total increase in area of a fibre type (Wang et al. 1993) or by multiplying fibre size by fibre proportions (Hikida et al. 2000). Wang et al. (1993) reported significant increases in the size of all fibre types following 18 weeks of resistance training. While this resulted in an increase in the total area taken up by the type I and IIA fibres, there was only a small and non-significant increase in total type IIX fibre area due to decreased proportions of this fibre type following the resistance training. Similarly, Hikida et al. (2000) noted increases in the size of all fibres but a decrease in type IIX contribution to the muscle due to a shift from the IIX to IIA fibres.

Effect of Resistance Training on Capillary Density

Similar to its effects on muscle oxidative capacity, it has been widely believed that there may be a dilution effect of resistance training on capillary density. Luthi et al. (1986) reported no change in either capillary to fibre ratio or capillary density (capillaries/mm^2) following a six week resistance training program. However, the training period used in this study was only six weeks long which may have been an insufficient length of time to determine any changes in capillary density. Tesch et al. (1984) compared muscle samples from weight and power lifters, endurance athletes and untrained controls and reported the capillary to fibre ratio to be similar between the resistance trained and untrained subjects although lower than the endurance athletes. However, the capillary density of the weight and power lifters was significantly lower than in either the endurance athletes or the untrained subjects. Green et al. (1998) reported an increase in the capillary to fibre ratio following 12 weeks of heavy resistance training in healthy young subjects. However, when the increase in fibre cross-

sectional area was taken into account there was no increase in capillary density. Frontera et al. (1990) reported increases in capillary to fibre ratio following 12 weeks of resistance training in 12 healthy elderly subjects however they did not investigate whether there were any changes in capillary density (capillaries/mm^2). Similarly, Hepple et al. (1997) reported increases in the capillary to fibre ratio following nine weeks of resistance training in healthy elderly subjects. However, although they found no increase in the number of capillaries per mm^2 of tissue, they did report an increase in the capillary to fibre perimeter exchange index, indicating a reduced capillary diffusion distance following nine weeks of resistance training in elderly male subjects.

Effects of Resistance Training on Mitochondrial Volume and Density.

It has been suggested that long-term resistance training may result in a dilution of mitochondrial volume and density in skeletal muscle due to muscle hypertrophy (MacDougall et al. 1979). Alway et al. (1988) showed that muscle mitochondrial volume was ~30% lower in both type I and II muscle fibres of strength-trained individuals compared with endurance trained athletes and control subjects. Luthi et al. (1986) reported that the absolute volume of mitochondria in muscle remained unchanged following a six week resistance training protocol, however, due to an increase in the volume of myofibrils (10%), the volume density of the mitochondria (volume of mitochondria per unit volume of muscle fibre) decreased by 9.6%. In contrast, Wang et al. (1993) reported that a group of women who performed 18 weeks of high-repetition resistance training underwent a significant increase in the absolute volume of mitochondria by 18%, 25% and 6% in muscle fibre type I, IIA and IIX respectively. When this result was indexed against myofibrillar changes however, no change in mitochondrial density was noted. Jubrias et al. (2001) studied the effect of 24 weeks of resistance training in a group of elderly subjects and reported a 31%

increase in volume density following the training protocol. However, they did not index these changes against muscle cross-sectional area.

Effects of Resistance Training on Enzymes of Oxidative and Glycolytic Metabolism

Costill et al. (1979) reported increases in the activity of the mitochondrial enzymes malate dehydrogenase (MDH) and SDH following seven weeks of resistance training. Similarly, Côté et al. (1988) demonstrated that the activity of the oxidative enzymes MDH, HAD and KGDH increased significantly as a result of ten weeks of repeated isokinetic strength training. Further, Wang et al. (1993) also showed that 18 weeks of resistance training resulted in an increase in the activity of COX. However, they noted no change in the enzyme activity of CK, CS, HAD, PFK or PHOSH. In addition Wang et al. (2000) reported no difference in either MAPR or a range of oxidative enzymes in young resistance trained compared with untrained controls. In contrast, Tesch et al. (1987) demonstrated that the activities of both hexokinase (HK) and creatine kinase (CK) decreased following six months of resistance training. Additionally, there was a trend towards a decrease in the activity of CS, PFK, LDH and myokinase (MK) over the course of the training study. Further examples of the effects of resistance training on oxidative and glycolytic enzyme activity are presented in Table 2.4.

Effects of Muscle Mass on Mitochondrial Volume Density and Oxidative Enzymes Activities

The conflicting findings in indicators of muscle oxidative capacity following resistance training may be due to differences in training protocols, including the length and intensity of these protocols, and the exercises used. Details of the effects of the resistance training protocols discussed on skeletal muscle size, fibre cross-sectional area and oxidative capacity

are listed in Table 2.4. Where resistance training induces muscle hypertrophy, these changes may mask any increases in total muscle oxidative capacity or capillarity. For example, a seven week study by Costill et al. (1979) reported increases in the activities of the enzymes MDH and SDH while Cote et al. (1988) reported similar results in the activities of MDH, β-HAD and KGDH following 10 weeks of training. Neither of these studies noted any skeletal muscle hypertrophy following the training intervention. Conversely Tesch et al. (1987) and Alway et al. (1988) reported significant muscle hypertrophy following six months and 10.6 years of heavy resistance training respectively with a trend towards decreases in the activities of a range of oxidative enzymes (Tesch et al. 1987) and decreases in mitochondrial volume density (Alway et al. 1988). In general, it can be seen from Table 2.4 that where resistance training results in muscle hypertrophy in young subjects, muscle mitochondrial volume density and oxidative enzyme activities are either unchanged or reduced. Elderly subjects, however, may experience an increase in muscle oxidative capacity following a resistance training program even after muscle hypertrophy is considered (Frontera et al. 1990). This may be due to these subjects starting from a lower base and hence, having greater scope for improvement.

2.5.4 Summary of Training Adaptations.

Endurance and resistance training cause adaptations in skeletal muscle. The primary adaptations that have been reported following endurance training include an increase in muscle oxidative capacity (Schantz et al. 1983; Staron et al. 1984; Wibom et al. 1992), increased capillary density (Denis et al. 1986; Coggan et al. 1992b) and a shift towards more oxidative muscle fibre proportions (Coyle et al. 1991; Proctor et al. 1995). Resistance training has also been demonstrated to result in a shift towards more oxidative fibre proportions (Green et al. 1998; Hikida et al. 2000) combined with increases in the cross-

sectional area of the muscle fibres (MacDougall et al. 1979; Frontera et al. 1990; Wang et al. 1993). While some studies have reported a dilution effect of the increases in muscle size on factors affecting oxidative capacity such as capillary density (Green et al. 1998) and muscle oxidative capacity (Wang et al. 1993), it appears that in elderly subjects, there may in fact be an increase in these factors following a resistance training intervention (Frontera et al. 1990).

Table 2.4. The Effects of Resistance Training on Muscle Fibre Type, Capillary Density and Oxidative Capacity

Source	Age(year) /Sex	Muscle	Training	CSA Changes (%)			TM	Metabolic Enzymes				Capillary Density		Mitochondria	
				I	IIA	IIX		CS	SDH	HAD	PFK	Cap/Fibre	Cap/mm^2	VD	V/M
(Costill et al. 1979)	23/M	VL	7w.	↔	↔	↔			↑ 11*						
(MacDougall et al. 1979)	22/M	TB	6mo.	ST ↑ 27*	FT ↑ 33*									↓ 26*	↓ 25*
(Staron et al. 1984)	24/M	VL	> 3 yrs.	↑ 12*	↑ 33*		↑ 9*							↓ 35* in I, IIA and IIX	
(Luthi et al. 1986)	18/M	VL	6w.				↑ 8							↔	↓ 10*
(Tesch et al. 1987)	26/M	VL	6 mo.	ST ↑ 4	FT ↑ 29*			↔			↓ 16				
(Alway et al. 1988)	17-32/M	TS	10.6 yrs.	↑*	↑*									↓36* in I and ↓29* in II	
(Cote et al. 1988)	24/M&F	VL	10w.	↔	↔	↔	↔			↑ 25*					
(Frontera et al. 1990)	60-72/M	VL	12w.		↑ 20*			↑ 36*				↑ 13*			
(Staron et al. 1991)	21/F	VL	20w.	↑ 17*	↑ 39*	↑ 47*									
(Wang et al. 1993)	20/F	VL	18w.	↑ 20*	↑ 29*	↑ 22*		↔		↔				↑ 18*, 24* & 6 in I, IIA & IIX	↔
				↑ 20*	↑ 25*	↑ 6	(AV)								
(Hepple et al. 1997)	68/M	VL	9w.		↑ 27*							↑ 18*	↔		
(Green et al. 1998)	19/M	VL	12w.	↑ 14*	↑ 24*	↑ 14*			↔			↑ 33*	↔		
(Hagerman et al. 2000)	64/M	VL	16w.									↑ 19	↔		
(Hikida et al. 2000)	64/M	VL	16w.	↑ 46*	↑ 34*	↑ 52*									
				↔	↑	↓	(AV)								
(Jubrias et al. 2001)	69/M&F	VL	24w.				↑ 9*							↑ 31*	

Changes = (RT – Controls)/controls x 100. VL, Vastus Lateralis; TS, Triceps Surae; VD, Volume Density; V/M, volume/myofibrils ratio; TM, thigh mass; ↑, increased; ↓, decreased; ↔, unchanged; * $p < 0.05$.

2.6 CHRONIC HEART FAILURE

Chronic heart failure (CHF) describes a life-threatening syndrome characterised by insufficient cardiac output. The major symptoms of CHF are fatigue on exertion and breathlessness (Clark et al. 1996). Myocardial damage caused by infarction results in poor ventricular contractility resulting in a reduced volume of blood being ejected from the ventricle during systole. Low left ventricular ejection fraction (LVEF) is associated with poor survival rates in CHF, but improved exercise tolerance is a better prognosticator of survival (Bittner et al. 1993). However, there is no relationship between LVEF and exercise tolerance expressed as $\dot{V}O_{2\,peak}$ in these patients (Cohn et al. 1993).

2.6.1. Pathology of Chronic Heart Failure

The majority of CHF cases fall into one of two main categories, namely, ischaemic and dilated cardiomyopathies. Ischaemic cardiomyopathy, a clinical sequela to coronary artery disease is characterised by local dyskinetic, akinetic or hypokinetic contraction and chronic arrhythmias that further compromise myocardial function. Ischaemic cardiomyopathy accounts for almost 80% of CHF cases. Dilated cardiomyopathy accounts for approximately 4% of patients with heart failure in the community (Poole-Wilson 1996b) and has a range of possible aetiologies. Almost one quarter of dilated cardiomyopathies are familial although the specific causes are not yet well understood. The remainder are due to idiopathies which include viral infections, toxins, immunological mechanisms and genetic disorders (Poole-Wilson 1996b).

2.6.2 Epidemiology of Chronic Heart Failure

Little reliable epidemiological data has been published that investigates the frequency of heart failure in the general population. Love et al. (Love et al. 1996) believe that 1-2% of the European and United States populations have heart failure and that the prevalence of the

disease rises sharply with age, affecting as much as 10% of the population of over 80 year olds. With the aging of the western population and the improvements in reducing the mortality rate in this disease, it is possible that the incidence of heart failure may increase by as much as 70% by the year 2010 (Ho et al. 1993). CHF is now the most common cause of non–elective hospital admissions in people over 60 years of age. There are a number of predisposing correctable risk factors that contribute to the prevalence of CHF in western populations, which include hypertension, diabetes, cigarette smoking, obesity and a high total to high–density lipoprotein (HDL) cholesterol ratio (Kannel and Belanger 1991).

2.6.2.1 Morbidity

There are a number of factors responsible for the deterioration of patients with CHF (Figure 2.9). These include:

a) Increased diastolic pressure (afterload) caused by chronic neurohormonal overactivity (eg. noradrenaline; Fig. 2.9a) resulting in widespread vasoconstriction and an increase in total peripheral resistance.

b) Fluid retention, which compromises a failing heart according to the Starling hypothesis (Fig. 2.9b).

c) Remodeling of the myocardium in response to catecholamines (Fig. 2.9c).

d) Skeletal muscle remodeling or atrophy in response to the activation of cytokines and neurohormones, disuse atrophy or recurrent ischaemia on exercise (Fig. 2.9d).

e) Cell apoptosis in response to destructive influences of cardiac cytokines and oxygen radicals (Fig. 2.9e).

f) Worsening of myocardial ischaemia (Fig. 2.9f).

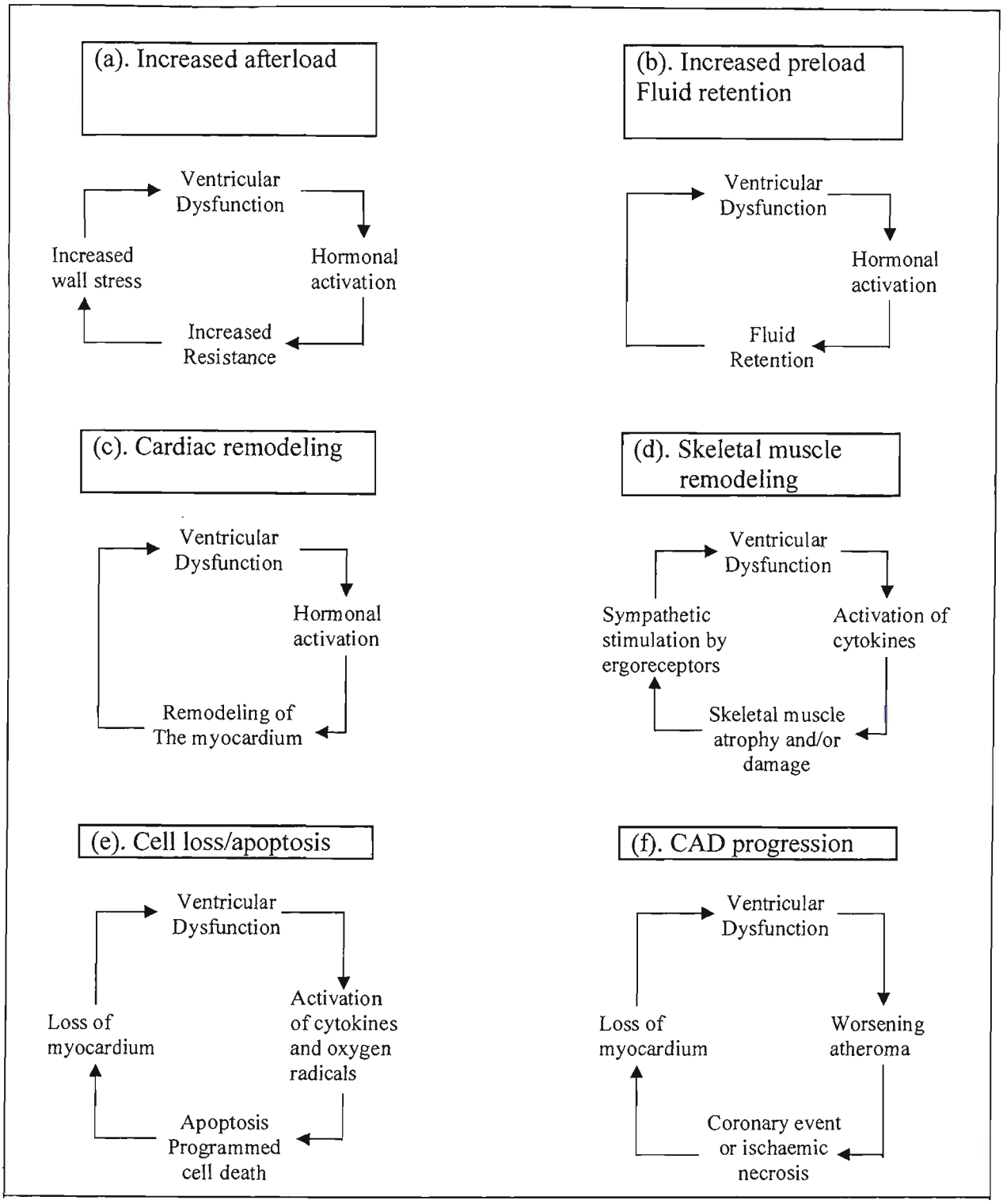
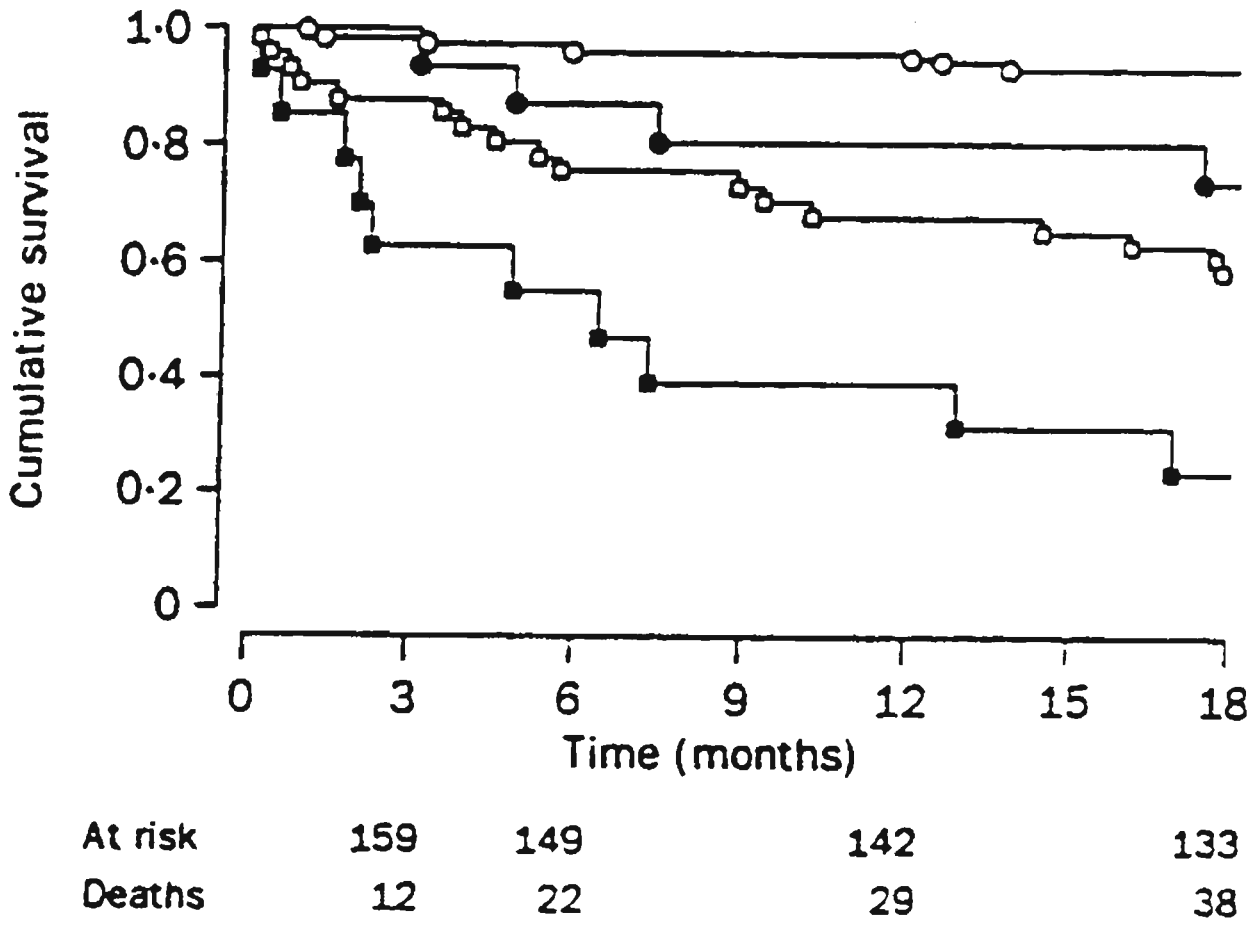


Figure 2.9. Spirals of Chronic Heart Failure. CAD = Coronary artery disease. (Adapted from (Poole-Wilson 1996a), p.41).

2.6.2.2 Mortality

CHF is associated with a high mortality rate, ranging from 50% at 5 years after diagnosis for patients selected from a general population to 50% in 1 year after diagnosis in those with advanced heart failure (Poole-Wilson 1993). The major risk factors or predictors of patient survival have been identified (Hammermeister et al. 1979; Anker et al. 1997c; Walsh et al. 1997). Hammermeister et al. (1979) examined the relationship between survival and 46 independent variables in ischaemic heart failure in 733 medically-treated and 1870 surgically-treated patients. They found that left ventricular end-systolic volume was the best predictor of mortality in the medically treated group, followed by age and stenotic lesions. In the surgically treated group, ejection fraction, stenotic lesions in the left main coronary artery and left ventricular contractility were found to be the most important predictors of survival. Due to exercise data being restricted to 46% of the population studied, the authors chose to leave these variables out of the final analysis. More recent studies (Anker et al. 1997c; Walsh et al. 1997) have included exercise data in their analyses. Using a much smaller sample (n=84) and over a shorter time frame (2 yr), Walsh et al. (1997) reported reduced daily activity levels measured with pedometers to be the most powerful predictor of mortality. They also reported that peak oxygen consumption ($\dot{V}O_{2\ peak}$) correlated negatively with survival. Anker et al. (1997c) found that peak oxygen consumption during incremental exercise ($\dot{V}O_{2\ peak}$) of less than $14ml.kg^{-1}.min^{-1}$ was a significant predictor of mortality in CHF. Other important predictors were a New York Heart Association (NYHA) functional class III or IV rating and a low time to fatigue during constant load exercise. Using the Kaplan–Meier survival analysis (Fig.2.10), the authors reported a survival rate of 93% of patients at 18 months with a VO_2 peak > 14 $ml.kg.^{-1}$ min^{-1} and no evidence of skeletal muscle cachexia. Conversely, the 18 month survival rate of patients with both of these risk

factors was found to be only 23% (n=13) while the 55 patients with only one of these risk factors had intermediate survival figures (62% at 10 months).



□ Non – cachectic and $\dot{V}O_{2\,peak} < 14$ ml.kg^{-1}.min^{-1} (n = 40, 17 deaths)

● Cachectic and $\dot{V}O_{2\,peak} \geq 14$ ml.kg^{-1}.min^{-1} (n = 15, 4 deaths)

■ Cachectic and $\dot{V}O_{2\,peak} < 14$ ml.kg^{-1}.min^{-1} (n = 13, 10 deaths)

O Non – cachectic and $\dot{V}O_{2\,peak} \geq 14$ ml.kg^{-1}.min^{-1} (n = 103, 7 deaths)

Figure 2.10. Kaplan – Meier survival and cumulative hazard curves for 18 month survival of 171 patients with CHF. (Adapted from (Anker et al. 1997c)).

2.7 CHF AND EXERCISE TOLERANCE

Patients with CHF cease exercising at lower work rates than do normal subjects. This occurs as a result of the early onset of fatigue and breathlessness, which are the major symptoms of

this condition (Clark et al. 1996). Hypotheses for this include insufficient cardiac output (Hammermeister et al. 1979; Franciosa et al. 1981), poor peripheral perfusion (Wilson et al. 1984) and histochemical and biochemical changes in the skeletal muscles (Hambrecht et al. 1997; Näveri et al. 1997).

Franciosa et al. (1981) found no correlation between exercise capacity and indexes of left ventricular function in 21 CHF patients leading the authors to conclude that changes in ventricular performance at rest do not appear to be the limiting factor in the functional capacity of CHF patients. This finding is supported by those of Cohn et al. (1993) who reported no relationship between resting LVEF and $\dot{V}O_{2\,peak}$ in CHF patients. Wilson et al. (1984) attempted to determine the relationship between skeletal muscle blood flow and maximal exercise capacity in CHF patients with a range of disease severity. CHF patients were divided into three groups according to their $\dot{V}O_{2\,peak}$. Leg O_2 extraction percentages and arteriovenous lactate differences were measured and found to be similar in all groups at the cessation of exhaustive exercise despite a progressive decline in maximal cardiac output, maximal leg blood flow and maximal leg $\dot{V}O_2$ as the severity of exercise intolerance increased. The results suggest that exercise was limited by blood flow to the exercising muscles in these patients. However, the authors could not discount the possibility that the exercise intolerance noted in the more severe patients was due to deconditioning or atrophy of skeletal muscle associated with an increase in cellular glycolysis.

Several studies have attempted to determine the role of skeletal muscle abnormalities in the decreased exercise tolerance reported in CHF patients. Yamani et al. (1995) investigated the mechanisms of exercise intolerance in 11 moderate CHF patients and 10 age-matched sedentary controls. They tested muscle endurance and found decreased muscular endurance

in CHF patients compared to controls but with no related increase in recovery time in these patients. This led them to conclude that exercise intolerance in CHF is due to the skeletal muscle abnormalities that have been reported in this condition. Joundeau et al. (1992) reported that $\dot{V}O_{2\,peak}$ in CHF patients is increased by adding arm exercise to a cycle exercise protocol. This finding was not replicated by the addition of arm exercise to cycle exercise in their seven age – matched control subjects. The implication of these findings is that it is not the capacity to deliver oxygen to the exercising muscles that is the cause of exercise intolerance in CHF but rather, it is the capacity of the exercising muscle to utilise oxygen (Drexler and Coats 1996).

2.8 SKELETAL MUSCLE AND EXERCISE TOLERANCE IN CHF.

In healthy subjects, a range of variables within the cardiovascular and muscular systems limits exercise tolerance. Similar mechanisms appear to also be the limiting factors in CHF, despite occurring at lower exercise intensities. Briefly, exercise intensity and duration are limited by the rate at which skeletal muscle mitochondria can use oxygen in the formation of ATP. At exercise intensities approaching maximum, the mitochondria are unable to supply the demand for ATP through aerobic processes. As a result, anaerobic mechanisms increase their supply of ATP to cope with demand. A major byproduct of anaerobic metabolism is lactic acid, which accumulates in the muscle during intense exercise causing an increase in muscle acidosis, which is possibly related to the process of fatigue.

The structural and metabolic changes that occur in skeletal muscle in response to CHF have been widely investigated (Mancini et al. 1989; Sullivan et al. 1990; Massie et al. 1996; Näveri et al. 1997) with researchers using a variety of techniques, including muscle biopsy and magnetic resonance to determine these changes. The following sections will discuss

these changes with the some of the most important papers and their major findings listed in Table 2.5.

2.8.1 Muscle Oxidative Function

A number of researchers have chosen to investigate the metabolic effects of an acute bout of exercise through the use of nuclear magnetic resonance spectroscopy (NMRS) (Wilson et al. 1985; Massie et al. 1988; Mancini et al. 1992; Toussaint et al. 1996). When exercise is sufficiently intense that aerobic mechanisms cannot meet the demand for ATP resynthesis, PCr is used at a more rapid rate than it can be replaced. Thus a low PCr:P*i* ratio means that a significant proportion of the energy is derived from ATP produced anaerobically. This is associated with a low intramuscular pH, suggesting increased lactic acid production. It has been found that there is a more rapid depletion of PCr reserves in CHF than in control subjects (Massie et al. 1987; Massie et al. 1988; Mancini et al. 1989). The rate of PCr resynthesis, a purely oxidative process (Kemp et al. 1993), has also been found to be lower in CHF patients (Mancini et al. 1989). Kemp et al. (1996) reported that the halftime for PCr resynthesis was elevated in CHF patients compared to healthy control subjects following forearm but not calf muscle exercise. However, similar mean differences were present between the groups in both muscles. Additionally, the researchers reported an increased rate of PCr utilisation in CHF patients when plotted against exercise $\dot{V}O_2$, suggesting a reduced ability of oxidative mechanisms to resynthesise ATP. This finding has also been reported elsewhere (Wilson et al. 1985; Weiner et al. 1986).

The major weakness with studies involving NMR spectroscopy is that, generally, they are confined to small muscle groups, which therefore examine the mechanism of local fatigue in small skeletal muscles. Consequently, these studies have not involved large enough muscle

masses to invoke a maximal circulatory and ventilatory response, which may have an effect on the overall fatigue mechanism. Okita et al. (1998) also used magnetic resonance spectroscopy to attempt to determine differences in muscle metabolic characteristics between CHF patients and healthy controls. However, unlike earlier studies, the investigators utilised systemic exercise which has not commonly been used in MRS studies because the bores of the magnets are not sufficiently wide to allow systemic exercise to be performed inside. The investigators circumvented this problem by exercising their subjects outside of the coil and applying a "so called" metabolic freeze protocol at fatigue. This method involved subjects performing maximal upright bicycle exercise until fatigue with an uninflated cuff around their thigh. At fatigue, the subjects ceased pedalling and the cuff was simultaneously inflated to a suprasystolic pressure preventing aerobic recovery of metabolites. Cuff pressure was maintained while subjects were transferred to the magnet where fatigue NMRS was immediately measured. The authors reported a greater decrease in muscle pH at the cessation of exercise in CHF patients and this was combined with lower blood lactates at peak exercise and following three minutes of recovery. Additionally PCr, depletion occurred more rapidly at equivalent workloads in the CHF group suggesting that impaired skeletal muscle metabolism was a major factor in fatigue during maximal systemic exercise, although this could also be due to impaired maximal muscle blood flow.

Muscle biopsy studies have examined the effect of CHF on muscle oxidative capacity by measuring the activities of a range of metabolic enzymes. Many studies have reported changes in the activities of some enzymes but not others (Sullivan et al. 1990; Duscha et al. 1999; Duscha et al. 2001; Schaufelberger et al. 2001). Unfortunately, the research is not clear on whether or not there is a decrease in metabolic enzyme activity as a result of CHF. This

section will review the findings that have been reported regarding metabolic alterations with CHF.

Changes in the level of certain muscle enzymes have been reported in relation to CHF (Mancini et al. 1989; Sullivan et al. 1990; Schaufelberger et al. 1996; Näveri et al. 1997). Ralston et al. (1991) reported lower activities of the oxidative enzymes CS, SDH and COX in CHF patients than in healthy controls. However, the age range of their control group was lower than that of the CHF patients. The discrepancy in ages may be at least partially responsible for the difference between the groups in this study, as age has been shown to effect muscle oxidative capacity (discussed section 2.5.1). However, other studies have also reported alterations in the activities of certain metabolic enzymes with CHF. Sullivan et al. (1990) compared 11 CHF patients with 9 age matched controls and found the activity of the oxidative enzyme SDH to be lower in the CHF patients. In comparison, PFK, a glycolytic enzyme, was found to be unaffected. This is in keeping with the findings of Näveri et al. (1997) who also reported no difference between PFK levels in CHF and control subjects. They did report decreased levels of KGDH (a Krebs cycle enzyme) and CPT, which plays an important role in fatty acid metabolism. KGDH activity correlated with $\dot{V}O_{2\,peak}$ in the whole study population and in the control subjects, highlighting the role of muscle oxidative capacity in exercise tolerance. However, no such correlation was reported in the CHF patients suggesting that factors other than the reduced muscle oxidative capacity may be responsible for the low $\dot{V}O_{2\,peak}$ in CHF.

Lipkin et al. (1988) examined a range of metabolic enzymes in nine stable NYHA functional class III CHF patients and compared their results to those previously reported in normal subjects. In contrast to previous studies, the investigators reported no differences between the

activities of the oxidative enzymes COX or succinate cytochrome reductase (SCR) with heart failure. A major problem with this study, however, is that the investigators did not recruit their own healthy subjects to compare against the CHF patients. Instead, they reported ranges of data for healthy subjects for each of the variables measured in their CHF group. No indication is given as to the source of the ranges of data reported for these healthy subjects or whether the ages or genders of these subjects match those of the CHF patients. Additionally, because ranges were used for the data from the healthy subjects, no meaningful statistics could be performed and all judgements regarding the data were made on a subjective basis. Two studies by Duscha and colleagues (Duscha et al. 1999; Duscha et al. 2002) have reported that there is no difference between CHF patients and control subjects in the activity of the enzyme CS, suggesting no difference between the groups in muscle oxidative capacity per unit of muscle weight. However, both these studies (Duscha et al. 1999; Duscha et al. 2002) reported that CHF patients had a lower activity of HAD, a key enzyme in β-oxidation, suggesting that the ability to metabolise fatty acids may be impaired in CHF. An earlier investigation (Mancini et al. 1989) also reported similar results in the activities of both HAD and CS. Opasich et al. (1996) reported no difference in the activities of the oxidative enzymes CS, SDH and COX between patients with severe CHF and healthy control subjects.

It is still unclear whether or not there is a reduction in skeletal muscle oxidative capacity with CHF. Most studies report lower oxidative enzyme activity in CHF patients than control subjects in at least one metabolic pathway. However, as shown in Table 2.5 several studies report no difference in the enzyme CS, while others report moderate to substantial reductions in the activity of this enzyme. Similar patterns exist for both SDH and HAD. The origin of these differences is not clear, however, the severity and duration of the CHF may be a contributing factor. A clear understanding of the role of muscle oxidative capacity in CHF is

further limited by the fact that the measurement of mitochondrial enzyme activity is, at best, an indirect indicator of muscle oxidative capacity as maximal enzyme activities are generally substantially in excess of *in vivo* maximal mitochondrial oxidative function (Letellier et al. 1994). Additionally, enzyme activities only provide information about single reactions in complex pathways. Mettauer et al. (2001), despite reporting a 40% lower CS activity in CHF patients compared to healthy sedentary controls, reported no difference in oxygen consumption in saponin treated muscle fibres. However the latter study only utilised a single group of substrates (glutamate and malate), and consequently, the observation is limited to a single metabolic pathway. To date, no studies have investigated the effect of CHF on MAPR which can be used to examine a more extensive range of metabolic pathways.

2.8.2 Capillary Density

A reduction in skeletal muscle capillary density is, potentially, a mechanism by which exercise intolerance may occur in CHF due to its implications for the potential reduction in skeletal muscle blood flow. As a result, a number of studies have investigated capillary density in CHF with generally consistent results. DeSousa et al. (2000) reported male rats with surgically induced heart failure to have a reduced capillary to fibre ratio when compared to sham operated animals. However, when the capillaries were indexed against the size of the muscle fibres (capillaries/mm^2) there was no difference between the two groups. Lipkin et al. (1988) also noted no difference between CHF patients and healthy control subjects in the number of capillaries per mm^2. However, there were serious limitations to this latter study which have been noted previously. Subsequent more robustly designed studies (Sullivan et al. 1990; Duscha et al. 1999) in humans with CHF have reported a reduced capillary to fibre ratio in CHF patients compared to healthy control subjects. However, only one of these studies (Sullivan et al. 1990) went on to investigate the number of capillaries per unit of area

and they reported no difference between the groups. With the exception of Lipkin et al. (1988), who had eight males and one female in their patient group, these studies were limited to male CHF patients. More recently, Duscha et al. (2002) investigated the effects of CHF on capillary density in both male and female CHF patients. Consistent with previous studies, the authors reported that there was a reduced capillary to fibre ratio in the male CHF patients when compared to healthy control subjects. However, the women with CHF were reported to have a tendency towards an increase in the capillary to fibre ratio when compared to healthy female subjects. Unfortunately, this study did not examine whether the female CHF group had a greater number of capillaries per mm^2 of muscle than their healthy counterparts.

In summary, it appears that in males at least, there is a reduction in the capillary to fibre ratio but that this does not result in an increase in the area of muscle serviced by each capillary. It is likely this is due to a decrease in muscle area and volume with CHF. This information is discussed in more detail in the following section.

2.8.3 Skeletal Muscle Fibre Type Proportions and Cross-Sectional Area.

Maladaptations in the morphology of the skeletal muscles have been widely investigated in CHF. Structural changes that have been reported in CHF include a decrease in the size and number of slow oxidative fibres with a concomitant increase in the number of fast glycolytic fibres (Mancini et al. 1989; Sullivan et al. 1990; Massie et al. 1996; Duscha et al. 1999; DeSousa et al. 2000). DeSousa et al. (2000) investigated the properties of skeletal muscle in an animal model of CHF. They reported a lower soleus weight in CHF compared to sham operated animals and a shift from oxidative to glycolytic fibres in the soleus muscle seven months after heart failure was induced. Sullivan et al. (1990) noted that CHF patients had a reduced proportion of type I muscle fibres and an elevated proportion of type IIX fibres

when compared to healthy control subjects. Similarly, Duscha et al. (1999) reported a higher percentage of MHCIIx proteins in heart failure patients compared to control subjects. A further study by Duscha et al.(2002) that compared the skeletal muscle of CHF patients and healthy control subjects matched for exercise tolerance ($\dot{V}O_{2\ peak}$) reported no differences in muscle fibre type proportions however. Lipkin et al. (1988) claimed that muscle atrophy was present in the type I and type II muscle fibres of three of the nine patients CHF patients that they investigated. However, as discussed in the section on oxidative capacity they had no control group and relied on subjective comparisons between their CHF patients and undisclosed control data. Sullivan et al. (1997) used electrophoretic methods to determine myosin heavy chain proportions in the vastus lateralis of a group of CHF patients and healthy control subjects. They reported that the CHF patients had a lower percentage of MHCI proteins and a higher percentage of MHCIIx proteins than the healthy subjects. In addition, they reported a significant correlation between MHCI percentage and $\dot{V}O_{2\ peak}$ in the CHF patients. Similarly, Vescovo et al. (2000) reported that CHF patients had a lower proportion of MHCI proteins in their skeletal muscle and a higher proportion of MHCIIx proteins with no change in MHCIIa proteins. Additionally the cross sectional area of the muscle fibres, was 15% lower in the CHF patients. The same group also examined the relationship between skeletal muscle myosin heavy chains (MHC's), NYHA functional class and $\dot{V}O_{2\ peak}$ in patients with CHF. They reported a negative correlation between functional class and MHCI while there was a positive correlation between functional class and MHCIIa and IIx. A positive correlation was also noted between $\dot{V}O_{2\ peak}$ and MHCI, while a negative correlation was reported between $\dot{V}O_{2\ peak}$ and MHCIIa and IIx suggesting that alterations in fibre type proportion may be responsible at least in part for the low exercise tolerance that is found in CHF.

Harrington et al. (1997) reported lower quadriceps and total leg muscle cross-sectional area in patients compared to healthy controls. Additionally, skeletal muscle cross-sectional area was significantly related to $\dot{V}O_{2\,peak}$ in CHF patients but not controls, supporting the hypothesis that it is primarily muscle wasting that reduces exercise tolerance in CHF. This conclusion was supported by the findings of Lang et al. (1997) who reported that lean leg mass correlated with $\dot{V}O_{2\,peak}$ in a group of 100 patients with stable CHF. More recently Cicoira et al. (2001b) investigated the relationship between skeletal muscle mass and exercise tolerance in a large group of stable, non-cachectic CHF patients. The researchers conducted a multivariate analysis comparing $\dot{V}O_{2\,peak}$ to a range of measured variables and reported that in their group of patients, $\dot{V}O_{2\,peak}$ (ml/min) was significantly related to appendicular lean mass, leading them to hypothesise that $\dot{V}O_{2\,peak}$ would increase following interventions aimed at increasing muscle mass. The findings of Schaufelberger et al. (2001) who reported that maximal exercise capacity was related to thigh muscle cross-sectional area further support this hypothesis.

2.8.4. Do deconditioning and/or aging explain muscle maladaptations in CHF?

Previously, it has been suggested that the changes that occur in skeletal muscle in CHF are similar to those that have been reported as a result of general deconditioning or aging (Minotti et al. 1993). However, Massie et al. (1996) believe that the muscle abnormalities in CHF patients were due to more than inactivity. CHF patients exhibit significant muscle atrophy while maintaining a reasonable level of muscle strength (Minotti et al. 1993; Massie et al. 1996). In contrast, disuse in normal sedentary subjects has been reported to result in greater relative decreases in strength than in muscle size (Minotti et al. 1993). Duscha et al. (2002) compared CHF patients and control subjects matched for $\dot{V}O_{2\,peak}$. HAD activity and

capillary density were lower in male CHF patients than sedentary controls. They conclude that deconditioning alone does not explain muscle abnormalities in CHF, at least in males.

Minotti et al. (1993) have reported normal aging to be associated with the selective atrophy of type II fibres and the relative preservation of type I fibres. In addition, it is well accepted that in healthy aging there is an increase in the proportions of oxidative type I and IIA muscle fibres at the expense of the glycolytic type IIX fibres (Kovanen and Suominen 1987; Frontera et al. 2000). This is the opposite to skeletal muscle in CHF where there is an increase in the proportions of type IIX muscle fibres at the expense of either type I (Sullivan et al. 1990; Massie et al. 1996) or type IIA fibres (Duscha et al. 1999; Schaufelberger et al. 2001).

2.8.5. Summary

It appears likely that the early onset of fatigue that occurs in CHF patients is at least partially due to skeletal muscle atrophy, resulting in a decreased muscle mass involved in the work. The reduction in muscle mass combined with alterations in muscle fibre proportions that may or may not result in reductions in metabolic capacity will result in an earlier onset of lactate production at any given workload. It appears unlikely from the literature that there is any reduction in capillary density in CHF, suggesting that impaired blood flow to the skeletal muscles is not an issue in the reduced exercise tolerance. However, it must be noted, that while reductions in capillary density will limit maximum potential blood flow and consequently, tissue gas exchange, there are a number of factors that have a role in the control of the degree of dilation or constriction of the capillary bed that have not been discussed in this section.

Table 2.5. Skeletal muscle maladaptations with CHF

Source	NYHA Class / LVEF	Muscle	Fibre Proportions			CSA (%)			Capillary Density		Muscle Metabolic Function						
			I	IIA	IIX	I	IIA	IIX	Cap/ Fibre	Cap/ mm^2	CS	SDH	HAD	PFK	LDH	O_2 Consump	PCr Resynthesis
(Lipkin et al. 1988)	3/ <45%	VL				↓*	↓*			↔							
(Mancini et al. 1989)	20 %	Gastroc			↑46 *		↓23*	↓30 *	↔	↔	↔		↓29*				
(Sullivan et al. 1990)	1-4 /21	VL	↓*	↔	↑*				↓*	↔	↓40*	↓39*	↓33*	↔			
(Ralston et al. 1991)	10-22%	VL									↓60*	↓90*					
(Massie et al. 1996)	2.6/24%	VL	↓*				↓*				↓*						
(Kemp et al. 1996)	2.2/28	Forearm															↓*
(Opasich et al. 1996)	3 / <30%	VL									↔	↔		↔	↔		
(Schaufelberger et al. 1996)	2-3 / <40%	VL									↓20*		↓20*				
(Duscha et al. 1999)	2-3 / <35%	VL			↑32 *				↓*		↔	↓23*	↓26*	↔	↔		
(Vescovo et al. 2000)			↓*	↔	↑*		↓*										
(Mettauer et al. 2001)	Class 3	VL									↓40*					↔	
(Duscha et al. 2002)	2-4 / <35%	VL	↔	↔	↔				↓* Males ↔ Females		↔		↓*				
(Schaufelberger et al. 2001)	3-4	VL						↑ ns	↓ ns	↔	↓*		↔		↔		

Changes = (CHF - Controls)/Controls x 100. VL, Vastus Lateralis; Gastroc, Gastrocnemius; ↑, increased; ↓, decreased; ↔, unchanged; ns, non-significant trend; * $p < 0.05$.

2.9. FACTORS INVOLVED IN MUSCLE ABNORMALITIES IN CHF

The mechanisms which are responsible for muscle abnormalities in CHF are unclear. As previously indicated, deconditioning alone is not sufficient to explain these abnormalities. A number of endocrine, paracrine and genetic factors have been implicated.

2.9.1. ACE Gene Polymorphism

Angiotensin converting enzyme (ACE) catalyzes the production of angiotensin II and renders bradykinin inactive. Both angiotensin II and bradykinin are involved in cardiovascular function via their contribution to the maintenance of vascular tone (Cambien et al. 1992) and may have a role in skeletal muscle metabolism by affecting the rate of substrate delivery (Woods et al. 2000). An ACE gene polymorphism in intron 16 is characterized by the presence (insertion - I) or absence (deletion - D) of the 287-bp sequence. Between 20 and 50% of the variation in serum ACE concentration may be due to this polymorphism, with the highest levels in individuals carrying the DD genotype (Rigat et al. 1992). Associations between the II genotype and prolonged bradykinin half-life (Murphey et al. 2000) and reduced angiotensin II production (Brown et al. 1998) have been described. The functional significance of these associations is that prolonged bradykinin half-life and reduced angiotensin II production would be expected to result in reduced arterial vasoconstriction and hence, reduced blood pressure. These changes might also improve substrate delivery to exercising muscle as a result of relatively enhanced endothelium-dependent arteriolar vasodilation in the II genotype compared with DD and ID (Woods et al. 2000) (discussed later).

Previously, the D allele has been associated with increased systolic blood pressure during exercise (Friedl et al. 1996), elevated ACE in left ventricular tissue (Danser et al. 1995) and left ventricular hypertrophy in elite athletes (Fatini et al. 2000) and Caucasian military

recruits (Montgomery et al. 1997). However, the relationship between the ACE D allele and pathological left ventricular hypertrophy is less certain (Lindpaintner et al. 1995).

ACE DD genotype has been related to an increased incidence of myocardial infarction (Cambien et al. 1992) and early onset coronary heart disease (Ruiz et al. 1994) independent of classical risk factors. Increased risk of secondary events following myocardial infarction have also been linked to the D allele (Yoshida et al. 1999). However, other studies (Lindpaintner et al. 1995) have reported no relationship between ACE gene polymorphism and heart disease. A meta analysis (Samani et al. 1996) has been conducted using data from 15 studies published between 1992 and 1996, containing a total of 3394 MI patients and 5479 control subjects. The analysis supported the proposition that the D allele confers an increased risk of MI with subjects with the DD genotype having 1.26 times the likelihood of suffering an MI compared to those with the II/ID genotypes. A possible source of error in this analysis, which was noted by the authors, was a possible publication bias in the studies with low subject numbers. The majority of these studies were almost all greater than the pooled estimate, leading the authors to suggest that small studies were only published if they reported a significant effect. Regardless of this, the majority of the published data suggests that the incidence of heart disease is greater in subjects with the D allele of the ACE gene (Cambien et al. 1992; Ruiz et al. 1994; Samani et al. 1996; Yoshida et al. 1999). Consequently, ACE gene polymorphism is worth investigating in CHF patients.

ACE genotype has also been related to exercise tolerance in healthy populations (Gayagay et al. 1998; Hagberg et al. 1998; Montgomery et al. 1998; Rankinen et al. 2000a; Rankinen et al. 2000b) and in pathological conditions (Abraham et al. 2002; Kanazawa et al. 2002). Previously, elite mountaineers (Montgomery et al. 1998) and rowers (Gayagay et al. 1998)

have been reported to have an increased incidence of the I allele than the general population. The incidence of the I allele has also been positively related to event distance in 91 British Olympic standard runners (Myerson et al. 1999). Ten weeks of physical training has also been reported to result in significantly greater improvement in repetitive elbow flexion in British army recruits who were homozygous for the I allele. However, a major weakness with many of these studies is that they contain relatively small numbers of subjects which increases the chance of a type II error. In contrast, other studies have found no relationship between ACE genotype and exercise tolerance when comparing athletes from a range of sports (Taylor et al. 1999; Rankinen et al. 2000b) against healthy control subjects or following an endurance training protocol in previously sedentary subjects (Rankinen et al. 2000a).

While the relationship between ACE genotype and exercise tolerance is still uncertain in healthy subjects, associations between $\dot{V}O_{2\,peak}$ and the ACE gene in pathological circumstances are becoming clearer. Abraham et al. (2002) found that CHF patients with the DD genotype had lower $VO_{2\,peak}$ than those with the II or ID genotype. Comparatively lower forced vital capacity, and breathing efficiency (increased $\dot{V}E / \dot{V}CO_2$) were also found in DD patients. Kanazawa et al. (2002) investigated 39 Japanese patients with chronic obstructive pulmonary disease and reported an association between the DD genotype, and a disturbance in peripheral tissue oxygenation during exercise, consistent with impaired diffusion of oxygen across the muscle micro-circulation. These findings are consistent with those of Hagberg et al. (1998) who observed a greater maximal a-vO_2 difference with higher $\dot{V}O_{2\,peak}$ in post-menopausal women of II genotype. Cardiac output was not different in these subjects, meaning the difference was entirely due to oxygen extraction by the muscle. Both coronary artery disease and left ventricular hypertrophy are associated with chronic heart failure (CHF)

and low exercise capacity is a strong and independent prognosticator in CHF (Bittner et al. 1993). Therefore, the ACE genotype may influence the etiology or severity of CHF and may also have a role in exercise tolerance and muscle function in this disease.

2.9.2 Tumor Necrosis Factor-α (TNF-α)

Tumor necrosis factor is a pleiotropic cytokine that at low concentrations acts as an important regulator of the inflammatory response by effecting paracrine or autocrine regulation of leukocytes and endothelial cells (Feldman et al. 2000). However, in higher concentrations, such as are found in many patients with CHF, TNF-α production exceeds the number of receptors on the cell surface, with excess TNF-α being released into the circulation where it exerts a range of effects including the initiation of metabolic wasting (Tracey et al. 1990; Feldman et al. 2000).

TNF-α is involved in the remodelling that occurs in cardiac muscle following infarction by stimulating the accumulation of macrophages and neutrophils at sites of inflammation (Krown et al. 1996) and may be produced by the cardiac cells themselves (Krown et al. 1996). By stimulating production of sphingosine, TNF-α appears to have a role in the initiation of apoptosis. This may be a favourable mechanism in the cardiomyocytes of CHF patients by limiting the area of cardiac cell involvement in remodelling following myocardial infarction (Krown et al. 1996). Torre-Amione et al. (1996b) reported elevated levels of TNF-α in the myocardium of end stage failing human hearts while non failing hearts do not express this protein. Additionally, they noted that protein levels of the two cell surface receptors TNFR1 and TNFR2 were reduced in the myocardial cells of end stage IHD and DCM patients. This observation suggests that over expression of TNF-α may be one of

several different maladaptive mechanisms responsible for the progressive cardiac decompensation that occurs in advanced heart failure.

Direct relationships have previously been reported between circulating concentrations of TNF-α and NYHA functional class (Torre-Amione et al. 1996), exercise intolerance (Cicoira et al. 2001a) and cachexia (Levine et al. 1990; Anker et al. 1997a). In addition, indicators of increased catabolism such as an elevated circulating concentration of cortisol (Anker et al. 1997a), elevated urea nitrogen (Levine et al. 1990), unintentional weight loss (Anker et al. 1997a), low BMI (Anker et al. 1997b) or low muscle cross sectional area (Cicoira et al. 2001a) have all been associated with elevated levels of circulating TNF-α in CHF patients. However as these studies were observational, it is possible that the increased TNF-α levels that were reported reflect the severity of the illness rather than influencing it.

A number of studies have investigated the effects of elevated levels of TNF-α in the systemic circulation in otherwise healthy animals. Fan et al. (1995) demonstrated that exogenous administration of TNF-α reduces circulating concentrations of both growth hormone and IGF-1 in fasted rats. Clearly, reductions in the circulating levels of these hormones (if also measured in muscle) without similar reductions in the circulating levels of catabolic hormones, such as cortisol, would alter the anabolic/catabolic balance leading to protein breakdown. Further work by Lang et al. (2002) has reported that 24 hours of continuous TNF-α infusion decreases *in vivo*-determined rates of protein synthesis in adult Sprague-Dawley rats. The authors believe that TNF-α impairs skeletal muscle and heart protein synthesis at least in part, by decreasing mRNA translational efficiency and that this causes a reduction in the synthesis of both myofibrillar and sarcoplasmic proteins. Interestingly, this study also reported that the infusion of TNF-α produced no change in

diaphragm protein synthesis rates and elevated protein synthesis rates in the liver and spleen. Lang et al. (1992) have also reported that elevated levels of TNF-α increases rates of glucose production and utilisation. In animals infused with TNF-α, hyperinsulinemia failed to increase glucose uptake by skin and blunted the insulin-mediated increase in muscle by 73%. Their results suggest that sustained elevations of circulating TNF-α, due to prolonged production, in patients and experimental animals with malignancies or infectious diseases, may be an important mechanism for the enhanced glucose flux as well as the insulin resistance seen in CHF.

Further studies examining the effect of TNF-α on muscle cell cultures have reported that TNF-α stimulates the activation and translocation of nuclear factor-κB (NF-κB) in skeletal muscle cells (Sen et al. 1997; Li and Reid 2000; Langen et al. 2001) by reducing the availability of glutathione, an antioxidant buffer (Sen et al. 1997). NF-κB appears to upregulate the activity of the ubiquitin proteasome pathway resulting in the acceleration of protein degradation (Li et al. 2003). The proposed cascade resulting in protein loss due to TNF-α is presented in Figure 2.11.

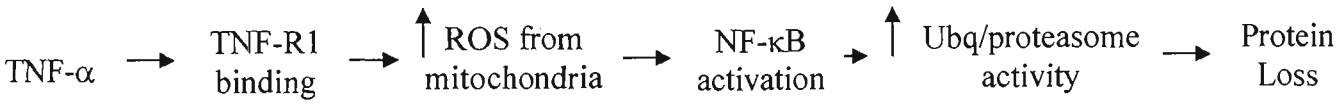


Figure 2.11. Proposed events regulating TNF-α induced muscle catabolism. Adapted from (Reid and Li 2001).

TNF-α is widely accepted as having a role in skeletal muscle wasting that has been observed in CHF. This may occur as a result of the direct effect of elevated levels of TNF-α on

skeletal muscle tissue initiating apoptosis or necrosis, may be due to the effect of circulating TNF-α on anabolic/catabolic balance or the effect of TNF-α on nitric oxide synthase (NOS).

2.9.3 Insulin Like Growth Factor

Insulin like growth factor–1 (IGF-1) is a single chained polypeptide that is structurally similar to insulin (Hameed et al. 2002). It is synthesised in the liver as a consequence of growth hormone (GH) secretion and delivered systemically, but it can also be produced locally in tissues such as skeletal muscle and this expression appears to be independent of GH (Hameed et al. 2002). Multiple isoforms of IGF-1 exist and specific isoforms appear to be targeted to specific tissues (Musaro et al. 2001). IGF-1 expression in skeletal muscle occurs when the muscle is subjected to mechanical stimulation and as a consequence this isoform of IGF-1 is commonly referred to as mechano growth factor (MGF). In addition, there are circulating proteins called IGF binding proteins (IGFBP) involved in the anabolic potential of IGF-1, as they modulate the availability of systemic IGF-1 by increasing the circulating half-life of IGF-1; mediating IGF-1 transport out of the circulation; and localizing IGF-1 in specific tissues (Clemmons 1993).

Incubation of muscle cell cultures with IGF-1 has been reported to stimulate cell hyperplasia and myofibre hypertrophy due to increased rates of protein synthesis and increases in the number of nuclei per unit of muscle length, respectively (Vandenburgh et al. 1991). Similarly, rat plantaris muscle subjected to continual overload via the excision of synergistic muscles has been reported to increase muscle mass, myofibre size, protein to body weight ratio and muscle IGF-1 peptide levels (Adams and Haddad 1996). The increase in IGF-1 was found to precede the hypertrophic response, suggesting a role for IGF-1 in muscle hypertrophy. Further evidence of a role for IGF in muscle growth comes in the form of a

study examining the over expression of MGF in transgenic mice. These animals have pronounced skeletal muscle hypertrophy and show signs of reduced susceptibility to the sarcopenia associated with aging (Musaro et al. 2001).

Single bouts of resistance exercise increase muscle IGF-1 mRNA expression in rats (Haddad and Adams 2002) and humans (Bamman et al. 2001). However, as these studies performed acute bouts of exercise they did not investigate the relationship between increases in muscle size and strength. Borst et al. (2001) examined the effects of 25 weeks of resistance training in healthy formerly sedentary male and female subjects and reported increases in circulating IGF-1. Further work by these authors, however, reported no change in resting serum concentrations of IGF-1, IGFBP-1 or IGFBP-3 despite increases in both strength and $\dot{V}O_{2\,peak}$ following resistance training in elderly subjects. The information that can be taken from either of these studies is limited as no attempt was made to determine changes in muscle mass as a result of training. As previously discussed, IGF-1 is also expressed within the muscle cells and there was no attempt to determine changes in this protein which may have a greater role in muscle hypertrophy than systemic IGF-1. Consequently, there is no evidence from resistance training studies directly linking increases in IGF-1 expression to remodelling (growth) of skeletal muscle following exercise.

Alterations in IGF-1 in CHF patients may have a role in the skeletal muscle maladaptations observed in this disease state. Previously, it has been reported that serum IGF-1 and IGFBP3 concentrations are lower in CHF patients compared to healthy control subjects (Osterziel et al. 2000; Anwar et al. 2002). However, Osterziel et al. (2000) only measured IGF-1 and IGFBP3 in a small (n = 20) group of CHF patients and compared their results to those obtained by others for normal subjects. Hambrecht et al. (2002) examined serum IGF-1

protein and skeletal muscle IGF-1 expression in 47 noncachectic male CHF patients and 15 age and gender matched controls. They found no difference between the groups in serum IGF-1, however, local muscle IGF-1 mRNA expression in the CHF patients was only half that of the control subjects. Quadriceps and total muscle area, as determined by computed tomography, were also reported to be significantly lower in the CHF patients than in the control subjects. A significant correlation was also noted between IGF-1 mRNA and thigh muscle cross-sectional area. These results indicate that there is a reduction in both systemic and muscle IGF-1 in CHF patients and that this may be at least partially responsible for the skeletal muscle maladaptations that have been reported in this disease.

2.9.4 The Nitric Oxide Synthases

Nitric oxide (NO) is an endogenous modulator of numerous cellular functions whose responses are mediated through a range of pathways (see Figure 2.12). It is involved in the regulation of muscle function by modulating glucose uptake, oxidative metabolism, muscle blood supply and contractility (Kaminski and Andrade 2001). NO is produced in reactions mediated by the enzyme nitric oxide synthase (NOS). Three isoforms of NOS have been reported in the literature; neuronal NOS (nNOS; type I), inducible NOS (iNOS; type II) and endothelial NOS (eNOS; type III). The three isoforms of NOS share a similar catalytic scheme whereby the amino acid L-arginine is used to form a variety of nitric oxide (NO) related molecules (Stamler and Meissner 2001). Many tissues, including skeletal and cardiac muscle, express one or more of these isoforms. NOS isoforms, expression, regulation and physiological roles are summarised in Table 2.6

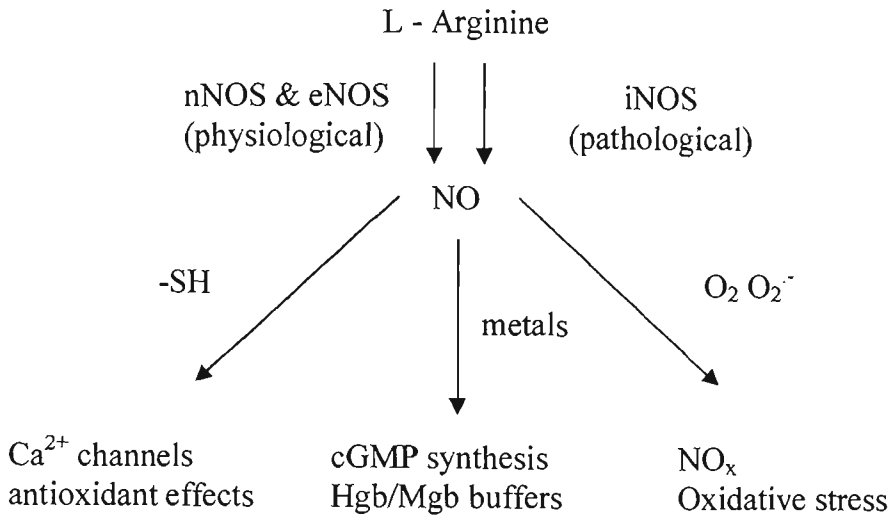


Figure 2.12 NO signalling pathways in skeletal muscle. Adapted from (Reid 1998). NOS, nitric oxide synthase; $O_2^{\cdot-}$, superoxide anion; -SH, reduced sulphydryl; NO_x, NO derivatives; cGMP, cyclic guanosine monophophate; Hgb, haemoglobin; Mgb, myoglobin.

Table 2.6. Nitric oxide synthase (NOS) isoforms in skeletal muscle. (Adapted from (Reid 1998))

NOS isoform	NNOS	iNOS	eNOS
Alternate Name	Neuronal; type I	Inducible; type II	Endothelial; type III
Expression	Constitutive	Inducible	Constitutive
Stimulus for Induction	Exercise	Inflammation	Exercise
Localisation	Sarcolemma	Cytosol	Mitochondria
Enzyme regulation	Ca^{2+} - calmodulin	Transcription	Ca^{2+} - calmodulin
Pattern of NO synthesis	Low, intermittent	High, continuous	Low, intermittent
Physiological Role	Mediate contractile function ? Cell-cell interactions ?	Antimicrobial action	Mediate mitochondrial respiration ?

Both nNOS and eNOS are expressed constitutively in low concentrations in skeletal muscle (Reid 1998). Syntheses of these isoforms are low and the calcium-calmodulin pathway controls regulation of function with the activity of these enzymes regulated by Ca^{2+} availability. In contrast, iNOS is not expressed constitutively by skeletal muscle but can be induced in this tissue in response to an inflammatory challenge. Consequently, the expression of iNOS in skeletal muscle is indicative of a pathological state. Unlike nNOS and

eNOS, calmodulin has a high affinity for iNOS meaning that the enzyme is active even at resting Ca^{2+} levels. Consequently, the expression of iNOS in skeletal muscle leads to elevated levels of NO in the muscle.

In healthy muscle, there is minimal expression of iNOS and the NO synthesised by nNOS and eNOS is likely to positively stimulate Ca^{2+} channels and have antioxidant effects, with any excess being buffered by myoglobin and haemoglobin. However, in a range of pathological states, iNOS expression is upregulated in skeletal muscle with an associated increase in NO production. Excess NO may not be buffered completely by the myoglobin and haemoglobin and may react with superoxide anions to generate reactive free radical species (Reid 1998).

2.9.4.1. Effects of NO in Skeletal Muscle

NO release from skeletal muscle is increased 50-200% during periods of repetitive isometric contraction (Balon and Nadler 1994). This occurs in response to the increased concentration of intracellular Ca^{2+} released from the sarcoplasmic reticulum (SR) and is a positive regulator of muscle function as NO promotes glucose transport and increases blood supply through its vasodilatory capabilities (Tidball et al. 1998). However, NO also appears to affect contractile function and metabolism in skeletal muscle cells.

NO appears to effect contractile function via two pathways and its effects may be dose dependant. NO binds to guanylate cyclase, activating the enzyme and raising cGMP levels in the muscle. Activation of guanylate cyclase increases cGMP which is a major positive regulator of shortening velocity in skeletal muscle (V_{max}) (Marechal and Beckers-Bleukx 1998). Cellular Ca^{2+} handling is also a potential site for NO influence of contraction via its role in regulating Ca^{2+} release from the SR. Low NO concentrations cause NO donors to

block oxidative modification of critical sulphydryl groups on calcium channels which inhibits their opening. Higher concentrations of NO donors induce oxidative cross-links between Ca^{2+} release channel subunits increasing the possibility of channel opening. NOS blockade has previously been demonstrated to reduce shortening velocity and decrease peak power (Morrison et al. 1996), suggesting that endogenous NO facilitates cross-bridge cycling, either through its effects on Ca^{2+} release or possibly via serving an antioxidant role, protecting regulatory sulphydryl groups on the myosin heavy chain from auto-oxidation by endogenous reactive oxygen species (ROS). In contrast, intravenous admininstration of a NO donor caused exaggerated declines in force production and perfusion pressure (Murrant et al. 1997), suggesting a multiphasic dose response curve. Regardless of whether the effects of NO on contractile function are positive or negative, they are small and may not be of major physiological significance (Kaminski and Andrade 2001).

NO can affect muscle metabolism via a number of mechanisms. It promotes glucose uptake while having an inhibitory effect on glycolysis, mitochondrial respiration and PCr breakdown (Reid 1998). High (submicromolar) concentrations of NO have also been demonstrated to rapidly and reversibly inhibit steady state turnover of isolated COX in brain synaptosomes *in vitro* (Brown and Cooper 1994). Alternatively Young {Young, 1998 #1057} reported that increases in cGMP via NO donors stimulate mitochondrial respiration. The nett effect of NO on metabolism therefore requires further investigation as it is possible that NO has substantially different effects at low versus high concentrations. Inhibition of mitochondrial respiration decreases ATP production and increases the cellular levels of ADP, AMP, GDP and *Pi*, which regulate a large range of cellular processes, including muscle contraction.

2.9.4.2. Chronic Effects of NO

It is possible that NO may have important long-term implications in skeletal muscle beyond the regulation of contractile force and muscle metabolism. Evidence exists linking an interaction between oxidative stress pathways and NO with the modulation of cytotoxic or cytoprotective effects. It is likely that elevated concentrations of NO within the cell will result in greater elevations in cytotoxic rather than cytoprotective effects. In contrast to the calcium dependent nNOS and eNOS, iNOS is expressed in immunologically activated cells in response to cytokines such as TNF-α (Riede et al. 1998). Increased levels of circulating TNF-α are common in CHF patients and the possible effects of this cytokine on skeletal muscle have been discussed in section 2.9.2. Consequently, muscle from patients with CHF (Riede et al. 1998; Adams et al. 1999) express much greater concentrations of iNOS than healthy muscle. Since iNOS is active at resting calcium levels elevations in the activity of this enzyme will potentially lead to increased NO concentration in the muscle. Indeed, patients with congestive heart failure have raised iNOS levels in their muscles leading to elevations in NO (Adams et al. 1997) and elevations in the local production of NO by iNOS following cytokine activation have been implicated in the muscle wasting of patients with advanced stages of heart failure and cachexia (Coats et al. 1994). Large increases in NO production within the cell results in increased production of reactive oxygen species and therefore possibly necrosis. NOS inhibitors also reduce muscle necrosis in inflammatory conditions, indicating a role for NO in necrosis.

It appears that NO is an important mediator of cell function in healthy muscle. However, in certain pathological states, NO production is dramatically increased in response to an increased expression of iNOS.

2.9.5 Anabolic and Catabolic Hormones

Cachexia in CHF is predictive of impaired prognosis independent of age, functional status, LVEF and peak oxygen consumption (Anker and Coats 1999) and is characterised by non-intentional weight loss. It is possible that the weight loss observed in cachectic CHF patients is due to hormonal changes causing an imbalance between protein synthesis and degradation at the cellular level. Consequently, research has been conducted investigating the effects of altered hormonal activation on anabolic/catabolic balance in CHF. Anker et al. (1997a) reported that plasma concentrations of the stress hormones adrenaline, noradrenaline and cortisol were all elevated in cachectic CHF patients compared to healthy control subjects. Human growth hormone (hGH) was also reported to be elevated in cachectic CHF patients compared to non-cachectic patients and control subjects. However, there was no accompanying increase in IGF-1 levels. While hGH is generally accepted as an anabolic agent, Anker et al. (1997a) argued that this is due to its role in stimulating IGF-1. Any elevation in hGH without a comparable elevation in IGF-1 would result in a shift towards catabolism. Consequently, the researchers presented the levels of circulating IGF-1 and hGH as a ratio and reported that the IGF-1/hGH ratio (anabolic/catabolic) was significantly reduced in cachectic CHF patients compared to non-cachectic patients and healthy control subjects. Further research by the same group (Anker et al. 1997b) has reported lower concentrations of dehydroepiandrosterone (DHEA), an anabolic steroid, in CHF patients compared to control subjects. Additionally, when catabolic and anabolic status were characterised by the log [cortisol]/log [DHEA] ratio this was found to be elevated in the CHF group with possible consequences for muscle wasting.

Previously, low intensity endurance training has been reported to have no effect on anabolic/catabolic ratios as defined by either testosterone/cortisol or DHEA/cortisol in CHF

patients (Kiilavuori et al. 1999). However, endurance training is not generally expected to result in shifts in anabolic/catabolic balance as evidenced by the lack of change in skeletal muscle mass with this form of training (Sullivan et al. 1995; Demirel et al. 1999; Scarpelli et al. 1999). To date, no study has investigated the effect of resistance training on anabolic/catabolic balance in CHF patients.

2.10 CHF AND EXERCISE TRAINING

As previously described, CHF patients exhibit reduced exercise tolerance that has been related to morbidity and mortality. In healthy subjects, exercise training results in improvements in exercise tolerance by both central and peripheral mechanisms. Similar effects in CHF patients would be expected to improve prognosis and quality of life.

2.10.1 Aerobic Training

The effects of deconditioning, due to a reduction in the volume of muscular work are reversed by aerobic or endurance training which has also been shown to improve local blood flow in healthy volunteers (Kingwell et al. 1997). The effects of this form of training on healthy subjects were discussed in an earlier section. In CHF patients, the level of exercise intolerance is strongly related to prognosis (Anker et al. 1997c; Walsh et al. 1997). As a result, many studies have examined the potential benefits of prescribing aerobic exercise as a mode of therapy in CHF.

Two to six months of aerobic exercise training has been demonstrated to increase $\dot{V}O_{2\ peak}$ (Sullivan et al. 1988; Coats et al. 1992; Ades et al. 1996) and exercise time to fatigue (Coats et al. 1992) in CHF patients. Several studies have reported increases in a-VO_2 difference and cardiac output at maximal exercise following endurance training (Sullivan et al. 1988;

Dubach et al. 1997) indicating improvements in peripheral oxygen delivery and uptake as a result of the training stimulus. However, the mechanism behind the increase in a-VO_2 difference seen in these studies was not investigated. Increases in 24 hour heart rate variability (Coats et al. 1992) and decreases in whole body noradrenaline spill over (Coats et al. 1992) and resting levels of angiotensin, aldosterone, vasopressin and atrial natriuretic peptide (Braith et al. 1999) have all been reported following exercise training. These observations are significant because neurohormonal mechanisms are thought to play a major role in the progression of CHF (Francis et al. 1984; Packer 1992). Some of the ways in which neurohormonal activation may act in the progression of CHF have been discussed in the section covering the pathology of CHF.

Many CHF patients suffer from skeletal muscle cachexia, which has previously been shown to involve the selective necrosis or atrophy of type I muscle fibres (Minotti et al. 1993; Massie et al. 1996) and a reduction in the activity of oxidative enzymes (Sullivan et al. 1988; Näveri et al. 1997). As previously discussed, skeletal muscle maladaptations are considered to be significant contributors to the exercise intolerance in CHF patients.

Ades et al. (1996) investigated the effect of a 12 week 3 hour/week aerobic exercise-training program in patients with coronary artery disease and reported a 16% increase in $\dot{V}O_{2\,peak}$ ($ml.kg^{-1}.min^{-1}$) after three months. In addition, the researchers took skeletal muscle biopsies from the vastus lateralis muscle of the training group at baseline and following 3 and 12 months of training. Following three months of training, they reported increases in the activity of the oxidative enzyme SDH and in capillary density, reflecting possible increases in muscle oxidative capacity and potential blood flow to the exercising muscles. Twelve months of exercise training resulted in a significant increase in the cross-sectional area of muscle fibres

although whether this was only in fibres of a specific type or across all fibre types was not specified. The authors concluded that physiological adaptations to aerobic exercise in older coronary patients are primarily localised to skeletal muscle and accordingly, training programs that focus primarily on skeletal muscle may be particularly valuable in these patients.

Several studies (Minotti et al. 1990; Cottin et al. 1996) have used NMR spectroscopy to determine the skeletal muscle response to endurance training. Minotti et al. (1990) reported a 260% increase in local muscular endurance and a decrease in the slope of P*i*/PCr versus work following a 28-day wrist flexor training program in 5 CHF patients. These adaptations were unaccompanied by any changes in blood flow, measured using venous occlusion plethysmography, either at rest or during exercise suggesting that the decreased P*i*/PCr slope was due to an increase in the oxidative capacity of the muscle fibres. These results are supported by the work of Adamopoulos et al. (1993) who used NMR to determine the effect of aerobic cycle training at 70-80% of maximal heart rate on muscle metabolism in CHF patients. They reported that following the training, PCr depletion during exercise was reduced and PCr recovery half times (an indicator of the maximal rate of mitochondrial ATP resynthesis) decreased significantly, although there was no significant change in the ADP recovery half time. Moreover, $\dot{V}O_{2\,peak}$ increased significantly following the training protocol, as did cardiac output at a set submaximal workload (50W). These improvements in metabolic function in the CHF patients were such, that at the end of the training protocol metabolic variables were similar between trained CHF and untrained healthy controls, suggesting that metabolism had returned to normal in the CHF patients. Despite the metabolic alterations however, the trained CHF patients had plantar flexion exercise times of less than 50% those of the healthy control subjects, indicating a lower local muscle exercise

tolerance than was the case in the healthy control subjects. It is possible that the lower time to fatigue in these patients compared to the healthy subjects may have been due to a lower muscle mass in the CHF patients compared to the control subjects. Muscle atrophy is a common maladaptation to CHF and has been discussed in a previous section.

The muscle biopsy technique has also been utilised by researchers attempting to determine the role of peripheral adaptations to improvements in exercise tolerance following endurance training in CHF. These studies have reported increases in the activities of certain oxidative (Hambrecht et al. 1997; Tyni-Lenne et al. 1997) and glycolytic (Tyni-Lenne et al. 1997; Kiilavuori et al. 2000) enzymes, increases in mitochondrial volume density (Belardinelli et al. 1995; Hambrecht et al. 1997), changes in capillary density (Scarpelli et al. 1999), and alterations in muscle fibre type proportions (Hambrecht et al. 1997). A recent study (Keteyian et al. 2003) on both males and females with CHF reported a significant increase in $\dot{V}O_{2\,peak}$ in male but not female patients after 14 to 24 weeks of aerobic exercise training. No differences were observed in muscle oxidative enzymes or capillary density. In the males however there was a significant increase in the MHC I isoform. These results clearly indicate a gender difference in responses to exercise training in CHF patients. The adaptations in skeletal muscle with aerobic training in CHF are summarised in Table 2.7. Only a single paper has reported increases in muscle fibre size following aerobic training in CHF patients (Belardinelli et al. 1995). However, there appeared to be a number of areas in which their data were difficult to reconcile. The authors reported no change in the capillary to muscle fibre ratio, coupled with an admittedly insignificant increase in the number of capillaries mm^2, despite an increase in the size of both type I and type II muscle fibres with training. A further investigation by some of the same researchers (Scarpelli et al. 1999) reported no change in muscle fibre diameters following an identical training intervention.

Table 2.7. Skeletal muscle adaptations to aerobic training in CHF

Source	NYHA Class / LVEF	Muscle	$\dot{V}O_2$ peak	Fibre Proportions			CSA (%)			Capillary Density		Muscle Metabolic Capacity					
												Enzyme Activities				Mitochondrial Density	
				I	IIA	IIX	I	IIA	IIX	Cap/Fibre	Cap/mm^2	CS	SDH	COX	PFK	VD	V/M
(Belardinelli et al. 1995)	II-III	VL		↔		↔	↑24*		↑17*	↔	↔					↑23*	
(Tyni-Lenne et al. 1997)	II-III	QF										↑44*			↔		
(Hambrecht et al. 1997)	II-III	VL	↑*	↑*		↓*								↑*		↑*	
(Scarpelli et al. 1999)	II-III	VL	↑*							↑13*							
(Tyni-Lenné et al. 1999)	II-III/ 28%	VL	↑20*	↓12*				↑21*									
(Kiilavuori et al. 2000)	II-III	QF		↔		↔	↔		↔	↔					↑*		
(Larsen et al. 2002)	II-III/ 33%	VL	↑*				↔	↔	↔								
(Keteyian et al. 2003)¥	21% M 34% F	VL	↑* M ↔ F	↑* M ↔ F	↔	↔				↔		↔		↔			

Changes = (CHF - Controls)/Controls x 100. VL, Vastus Lateralis; QF, Quadriceps Femoris; M, Males; F, Females; ¥, muscle fibre proportions based on MHC analysis;↑, increased; ↓, decreased; ↔, unchanged; * $p < 0.05$.

2.10.2 Resistance Training

While muscle abnormalities, such as decreases in the size and number of slow oxidative muscle fibres (Mancini et al. 1989; Sullivan et al. 1990; Massie et al. 1996) and a decrease in the oxidative enzymes (Sullivan et al. 1990; Näveri et al. 1997) have been reported in CHF, it appears that the largest factor involved in the early onset of fatigue in the skeletal muscle of CHF patients may be the decreased muscle mass involved in the work (Harrington et al. 1997). Resistance training results in increases in muscle size and strength in healthy young (Katch et al. 1985; Higbie et al. 1996) and elderly subjects (Frontera et al. 1990; Hikida et al. 2000). In elderly subjects, resistance training has also been demonstrated to increase endurance exercise capacity ($\dot{V}O_{2\,peak}$) in conjunction with increases in skeletal muscle oxidative capacity (Frontera et al. 1990) and in the capillary to fibre ratio (Hepple et al. 1997). If the same results were to occur following resistance training in CHF, the significant improvements in exercise tolerance in these patients could be expected to result in an improved quality of life and reduced rates of morbidity and mortality.

Resistance training as an intervention in CHF is a relatively new concept. The benefits of an aerobic training program for these patients are well established (Sullivan et al. 1988; Coats et al. 1992; Adamopoulos et al. 1993; Belardinelli et al. 1995; Ades et al. 1996; Demopoulos et al. 1997). However, few studies have yet attempted to measure the effects of a resistance training program in CHF patients. For many years it was believed that resistance training was contraindicated in CHF due to the increase in systolic blood pressure (afterload) that has been reported in this form of exercise. It was feared that the increased load on the heart in this situation may be dangerous in CHF patients, since an early study of isometric exercise, (as

distinct from dynamic resistance exercise), reported that left ventricular function was compromised in functionally limited patients who also had ischaemic heart disease (Kivowitz et al. 1971). Previous studies have reported no adverse cardiovascular events as a result of combining resistance training with aerobic training in post acute myocardial infarction (Daub et al. 1996) and stable coronary artery disease (Kelemen et al. 1986; McCartney et al. 1991) patients. These studies had lengths of 10-12 weeks and reported strength gains of between 10.5 and 29%. In addition, Kelemen et al. (1986) reported that their combined resistance and aerobic training group improved their time to exhaustion on the treadmill, whereas the aerobic training group did not, indicating that impaired exercise performance may be at least partly due to decreased leg strength. Featherstone et al. (1993) investigated the effect of acute bouts of heavy resistance weight lifting on a small number of men with stable CAD and reported no symptoms or electrocardiographic evidence of ischemia during the weight lifting, whereas 5 of the 12 subjects demonstrated electrocardiographic evidence of ischaemia during maximal treadmill exercise. These results led the authors to conclude that maximal repetition weight lifting at up to 100% of maximal voluntary contraction (MVC) appears to require a lower myocardial oxygen supply-to-demand balance than maximal treadmill exercise thereby making it a safer form of exercise training for cardiac patients than maximal treadmill exercise.

More recently, studies have been performed investigating the effects of resistance training on haemodynamic variables in CHF patients. Barnard et al. (2000) have reported systolic blood pressure to be no different between CHF patients tested during aerobic cycle ergometer exercise and immediately following performance of a dynamic weight lift at 1 repetition maximum (RM), either before or following a combined aerobic and resistance training program. Further

evidence supporting the safety of resistance training in CHF include an eleven week uncontrolled resistance training study of nine moderate CHF patients who trained using a hydraulic resistance system with activities of 30-60sec, interrupted by 1-2 minutes of stationary cycling, arm cycling and stair climbing. No adverse cardiovascular effects were reported during any of the nearly 300 training sessions that took place in this study (Hare et al. 1999).

Hare et al. (1999) conducted an eleven-week uncontrolled low intensity resistance training study of nine moderate CHF patients using hydraulic resistance equipment and reported increases in muscle strength and basal forearm blood flow. While there was no change in $\dot{V}O_{2\ peak}$, oxygen consumption ($ml.kg^{-1}.min^{-1}$) was significantly reduced for the full range of sub-maximal workloads following training. However, this study only measured gross endpoints, so the mechanistic factors behind the improvement are unclear. More recently, Pu et al. (2001) conducted a controlled study examining the effect of resistance training (RT) versus controlled stretching on exercise tolerance and skeletal muscle characteristics in 16 female NYHA class I-III CHF patients. The nine subjects randomised to the RT group trained three days per week for 10 weeks on pneumatic exercise equipment at 80% of 1RM while those randomised to the placebo group participated in low intensity stretching exercises on two days per week. Following training, the RT subjects had improved muscle strength and endurance and improved six minute walk distances. However, there was no change in $\dot{V}O_{2\ peak}$. The authors reported trends towards an increase in both the cross-sectional area of the type I fibres and muscle oxidative capacity as indicated by citrate synthase (CS) activity, both of which were independently predictive of the improved six minute walk distances. These changes may also be

expected to lead to improvements in $\dot{V}O_{2\ peak}$, although no such change was reported in this study.

While no study investigating the effects of RT alone has reported significant improvements in $\dot{V}O_{2\ peak}$, in CHF patients, improvements have been reported in submaximal indicators of exercise tolerance (Hare et al. 1999; Pu et al. 2001). Additionally, there are indications that this form of training may be useful in the reversal of the muscle atrophy reported in CHF. However, to date, the studies of RT in CHF have been limited by the absence of a control group or gender factors. Consequently, more research is required before conclusive decisions can be formed regarding the value of resistance training in CHF.

2.10.3 Combined Resistance and Aerobic Training Protocols.

Recently a number of studies have investigated the effects of combined resistance and aerobic training protocols of eight to twelve weeks duration in patients with CHF (Barnard et al. 2000; Maiorana et al. 2000a; Maiorana et al. 2000b; Beniaminovitz et al. 2002; Swank et al. 2002). Magnusson et al. (1996) compared the effects of eight weeks of single leg endurance training, with combined strength and endurance training in the contralateral leg in a small group (n = 6) of CHF patients. They compared these results with another group of CHF patients (n = 5) who underwent single leg strength training with the contralateral leg used as an untrained control. The authors reported significant increases in muscle fibre areas in the strength trained legs when data from all legs that underwent strength training were combined and compared to the non-strength trained legs. While strength training alone did not result in improvements in the capillary to fibre ratio, significant increases were reported in both the endurance leg and the

combined strength and endurance leg. Likewise, the activities of the oxidative enzymes, CS and HAD, were increased in muscle from the legs that underwent endurance training but not the leg that underwent strength training alone. These results indicate a role for both strength and endurance training in rehabilitation in CHF patients.

Maiorana and colleagues (Maiorana et al. 2000a; Maiorana et al. 2000b) used a circuit training protocol where patients moved through a series of seven resistance interspersed with eight aerobic exercise stations, spending 45 seconds at each and allowing 15 seconds to move from one station to the next. The authors reported (Maiorana et al. 2000a) significant increases in $\dot{V}O_{2\ peak}$, exercise test duration and muscle strength following the training protocol. In a follow up paper (Maiorana et al. 2000b), the authors also reported increases in peak reactive hyperaemic flow and forearm blood flow responses to infusions of acetylcholine and sodium nitroprusside in both the infused and non-infused limbs following the exercise training protocol. No difference was found in resting forearm blood flow following training compared to baseline measurements. These results suggest an improved vascular function and peak vasodilator capacity in patients with CHF. Unfortunately, these studies did not investigate the effects of the training on skeletal muscle morphology or function.

Barnard et al. (2000) compared the effects of a combined resistance and aerobic training protocol to aerobic training alone in CHF patients. In this study patients randomised to the aerobic training alone performed 30 minutes of aerobic exercise three times per week while those randomised to the combined training performed the same aerobic exercise and in addition, undertook two high intensity strength training (80% of 1 repetition maximum; RM) sessions per

week. The authors reported significant increases in muscle strength in the combined training group but not the aerobic training group following the training protocol. Significantly no adverse haemodynamic effects were reported in the combined training group following the training intervention. A follow up study by Swank et al. (2002) reported the effects of the combined aerobic and resistance training on quality of life measures in a subgroup of the CHF patients. Following the training protocol, the patients reported that the limitations on their daily activities imposed by the CHF had decreased and that the symptoms they associated with CHF, such as fatigue, loss of energy and dyspnea, had also decreased. However, because this study was not controlled, it is possible that the improvements in quality of life measures reported by the authors may have been solely due to the aerobic training portion of the exercise protocol.

A recent study by Beniaminovitz et al. (2002) investigated the effect of low intensity leg muscle training on muscle strength, $\dot{V}O_{2\,peak}$ and respiratory variables in a group of CHF patients. Training subjects exercised three times per week with 15 minutes of low-level aerobic exercise at a workload corresponding to 50% $\dot{V}O_{2\,peak}$ on both a bicycle ergometer and a treadmill. In addition to the aerobic exercise, subjects performed a series of leg callisthenics at each session which included hip flexion and extension using ankle weights and thigh muscle contractions using therabands. The authors reported improvements in thigh muscle strength and endurance following the three-month training protocol. In addition, $\dot{V}O_{2\,peak}$ and oxygen consumption at the anaerobic threshold were both increased in the training group, but not the active control group, following the exercise intervention. Although respiratory muscle strength and endurance were unaltered following the intervention in either group, the training group reported significant

improvements in the quality of life scores that were measured and were significantly different from the active control group on the Traditional Dyspnea Scale at endpoint.

The results of these studies suggest a role for combined training protocols in CHF rehabilitation. Unfortunately, no study has yet examined the effect of such an intervention on skeletal muscle morphology or oxidative function in CHF patients. While it is likely that there are improvements in muscle oxidative capacity and capillary density similar to those reported in ET studies following combined RT and ET protocols, these variables have not been measured. Additionally, muscle strength increases following a combined training protocol and may be related to the increases in muscle fibre area and muscle mass that have been reported following RT in CHF. However, until more studies are performed investigating the reasons for these improvements the mechanisms responsible will remain unclear.

2.10.4 Summary of training adaptations in CHF

Exercise training partially reverses the exercise intolerance that has been reported in CHF patients. Aerobic training results in improvements in $\dot{V}O_{2\,peak}$ and in muscle metabolic function. However, the effects of this form of training on muscle morphology are less clear. Resistance training has not been investigated as thoroughly as aerobic training. However, the studies that have utilised this form of training have reported increases in exercise tolerance and trends towards increases in metabolic function and muscle fibre size. Consequently, there is evidence to suggest a role for resistance training in CHF patients. A combination of aerobic and resistance training may yet prove to be the optimal form of training in the rehabilitation of CHF patients.

2.11. SUMMARY

Exercise tolerance is inversely related to prognosis in CHF. Maladaptations in the skeletal muscle of patients with CHF play a major role in the reduction in exercise capacity in CHF. These maladaptations include reductions in muscle oxidative function, alterations in muscle fibre type proportions and decreases in capillary density and muscle cross-sectional area. Although many of these maladaptations are well described, the mechanisms behind them are not well understood. A number of potential mechanisms have been implicated but to date the results of studies investigating these mechanisms have been inconclusive. Exercise training has been shown to improve prognosis and reverse many of the skeletal muscle maladaptations to CHF. However, while exercise is well accepted as a method of rehabilitation in CHF patients the optimal type of exercise is yet to be determined.

CHAPTER 3. GENERAL METHODS

3.1 OVERVIEW

This thesis comprises four studies as follows:

- Study I. The relationship between exercise limitation in chronic heart failure and changes in skeletal muscle structure and oxidative capacity (chapter 4).

The first study investigated mitochondrial ATP production rate (MAPR), metabolic enzyme activity, muscle fibre type and fibre size and capillary density in a group of moderate CHF patients and sedentary age matched controls. The study examined the relationships between these factors and exercise tolerance in the two subject groups.

- Study II. The effects of an 11 week resistance training program on skeletal muscle parameters in chronic heart failure (chapter 5).

The second study investigated the effects of a short-term resistance training program on a range of metabolic (MAPR, metabolic enzymes) and structural components (muscle fibre type proportions, muscle fibre size and capillary density) in a group of chronic heart failure patients.

- Study III. The role of chemical messengers on exercise tolerance and skeletal muscle adaptations in chronic heart failure (chapter 6).

The third study investigated a range of factors that may mediate exercise and skeletal muscle responses in CHF patients. The factors investigated included hormonal (cortisol and testosterone), cytokine (tumor necrosis factor - α) and Nitric Oxide Synthase (NOS) levels.

Levels of these factors were measured from plasma (cortisol, testosterone and TNF-α) and skeletal muscle (nNOS and iNOS) samples. Further, changes in these molecules were compared to changes in exercise tolerance and skeletal muscle over the course of a resistance training program.

- Study IV. An investigation into the effect of ACE genotype on exercise tolerance in CHF patients (chapter 7).

The fourth study investigated the role of ACE genotype on exercise tolerance in CHF patients. Patient responses to 11 weeks of resistance training were also compared on the basis of ACE genotype.

3.2 EXERCISE TESTS

All exercise tests, muscle strength tests and exercise training sessions were conducted at the Austin and Repatriation Medical Centre. Subjects in all studies performed exercise tests designed to measure peak aerobic power ($\dot{V}O_{2\,peak}$) and muscular strength and endurance. These tests were used to contrast exercise performance and muscular strength and endurance between the subject groups. Subjects performed each test at least twice, undergoing a familiarity trial either on the day of the test (some strength test subjects only) or approximately one week prior to baseline testing.

3.2.1 Measurement of Peak Aerobic Power

$\dot{V}O_{2\,peak}$ 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 $W.min^{-1}$. With the healthy controls the exercise test using the same equipment, commenced at 20 W and increased by 20 $W.min^{-1}$ until an RER of 1 was reached. Workload then increased by 10 $W.min^{-1}$ until volitional exhaustion or a level of 17 on the Borg scale of perceived exertion of 6-20 was reached. Measurements were made every 30 seconds of $\dot{V}O_2$ (OM-11 Medical Gas Analyser, Beckman, CA, USA), carbon dioxide production ($\dot{V}CO_2$) (LB2 Medical Gas Analyser, Beckman, CA, USA), and ventilation ($\dot{V}_E$ [BTPS], 47304A respiratory transducer with Fleisch pneumotach, Hewlett Packard, USA). Respiratory gas analysers were calibrated using a two point calibration procedure involving room air and gas cylinders containing β-gases in the range of 12-15% O_2 and 4-5% CO_2. β-gases had been previously checked against an α standard gas. A 3 litre calibration syringe was used to calibrate respiratory volumes. ECG was monitored continuously (EK43 Multiscriptor 12 lead ECG, Hellige, Belgium) and subjects' perception of exercise related symptoms was monitored via the Borg scale [Borg, 1973 #141]. Measurements of blood pressure, heart rate, arterial oxygen saturation (pulse oximetry; Biox 3700 Pulse Oximeter, Oxi-Radiometer, Boulder, Colorado) and respiratory exchange ratio (RER = $\dot{V}CO_2 / \dot{V}O_2$) were made each minute.

3.2.2 Measurement of muscular strength

Muscle strength was assessed using an isokinetic dynamometer (MERAC, Universal, Cedar Rapids, Iowa, USA) with microprocessor as has been described previously (Hare et al. 1999; Selig et al. 2002). Upper body strength was assessed using the shoulder push-pull manoeuvre, with strength determined as the peak torque produced in 3 consecutive maximal repetitions at an angular velocity of $60^{\circ}.s^{-1}$. The lower body was assessed similarly, using the knee extension-flexion manoeuvre at $60^{\circ}.s^{-1}$. A recovery period of at least 3 minutes was allowed between each

of the manoeuvres. Limb position and a torque correction for movement weight were performed just prior to each movement pattern.

3.3 RESISTANCE TRAINING PROTOCOL

The resistance training protocol was an 11 week controlled protocol with subjects randomised to the control group encouraged to maintain but not increase normal activity levels. Resistance exercise consisted of a range of exercises using a hydraulic resistance system (Hydra Fitness, Henley International, Belton, Texas) with 3 activities (chest push-pull; knee extension-flexion; shoulder push-pull), each of 30-second duration. These exercises were interrupted by 0.5 to 2 minutes of stair climbing, stationary cycling and arm cycling, at intensities sufficient to invoke recruitment of all muscle fibres. Stair climbing involved stepping up and down a set of wooden stairs and as the patients improved, hand weights were added to increase the training stimulus. Patients were repeatedly encouraged to maintain the maximal intensity they could maintain for the prescribed time during the arm and leg cycling exercises. Intensity was stressed as being more important than duration. Heart rates were monitored to ensure that the patients were working at a sufficient intensity to meet the requirements to be classified as resistance training in all exercises. A copy of the daily training diary with the list of exercises and the order in which they were performed is presented in appendix I. Subjects rested between each station until heart rate had dropped to within 10 beats of that recorded prior to beginning the exercise session. The number of circuits and the resistance settings were gradually increased according to a standardised protocol during the 11 weeks of training. CHF patients were monitored during exercise for adverse cardiovascular signs and symptoms by continuous electrocardiography and visual observation.

3.4 BLOOD ANALYSES

3.4.1. Blood Sampling

Blood samples were obtained from a dorsal hand vein via a 20-guage indwelling catheter. The catheter was attached to an extending line, with a three-way tap allowing multiple samples to be taken. The hand was protected via the use of a plastic cover (Tegaderm™), a common surgical glove and finally a veterinary glove that covered the length of the forearm. Arterialisation was maintained by immersion of the hand in a water bath maintained at 45°C. Approximately 2mL 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 (SaO_2) and plasma lactate concentration. Two syringes of blood were drawn at each sampling time, the first for blood gas analyses and the second for measurement of blood lactate. An additional 10mL of blood was drawn at rest for analyses of a range of signalling molecules. The method used for determination of these molecules will be detailed in chapter 6. To maintain catheter patency the line was flushed with heparinised saline (1-2mL) between each sample. Oxygen saturation in the blood samples was consistently in excess of 95% confirming arterialisation.

3.4.2. Blood Processing

The blood gas syringe was tightly capped and placed on ice for later analyses of oxygen partial pressure (PO_2) and partial pressure of carbon dioxide (PCO_2) which were determined using a model 865 Blood Gas Analyser (Ciba-Corning, Medfield, MA, USA). Blood samples from the second syringe were spun in a centrifuge and plasma lactate levels were determined using Roche Diagnostic Kit (Product No. 1822837) reagents. In short hydrogen peroxide is generated in an enzymatic reaction converting lactate to pyruvate. Peroxidase is then used to generate a coloured

dye using the hydrogen peroxide formed in the first reaction (Trinder 1969; Barhan and Trinder 1972). Intensity of the colour formed is proportional to the L-lactate concentration. The colour intensity of the formed dye was read using a Hitachi 917 Automated Clinical Chemistry Analyser.

3.5 MUSCLE SAMPLES AND ANALYSES

3.5.1 Vastus Lateralis Muscle Needle Biopsies

Muscle biopsy samples were obtained from the vastus lateralis muscle using the method originally described by Bergstrom (1962). Briefly, with the subject resting supine, the skin was prepared with a 10% povidone-iodine solution (Betadine, Faulding Pharmaceuticals, Adelaide, Australia). Lignocaine (1%) local anaesthetic (Xylocaine, Astra Pharmaceuticals, Sydney, Australia) was injected into the skin, subcutaneous tissue and muscle fascia. A single small incision was made in the skin. A 5-mm biopsy needle modified for suction was used to obtain 100-130mg of skeletal muscle. One portion of muscle (15-20mg) was prepared for fibre type analysis. This was achieved by embedding the muscle in an O.C.T. mounting medium (Tissue-Tek®) which was immediately immersed in isopentane cooled in liquid nitrogen (LN_2). The frozen sample was subsequently stored in LN_2. Two further pieces of muscle were immediately frozen in LN_2 and stored in LN_2 for later analyses of metabolic enzyme activities and muscle iNOS and nNOS. The remaining piece of fresh muscle (25-50mg) was placed on ice and used for the determination of MAPR.

3.5.2 Muscle Analyses

All muscle analyses with the exception of muscle iNOS and nNOS expression were conducted in the Exercise Metabolism Unit Laboratory, School of Biomedical Sciences, Victoria University of Technology. Muscle iNOS and nNOS expression analyses were conducted in the Clinical Physiology Laboratory at the Baker Heart Research Institute.

3.5.2.1 Muscle Fibre Types

For muscle fibre type determinations serial transverse sections (10μm), of the previously mounted muscle tissue, were cut in a cryostat (Microm GMBH D-6900 500, Heidelberg, Germany) 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 fibres as has previously been described (Brooke and Kaiser 1970). Cross-sectional area measurements of specific fibre types were made using a computerised imaging system (Analytical Imaging System, Imaging Research Inc, 1999). The mean cross-sectional area (μm^2) of each fibre type was computed based on 20 contiguous fibre areas per type, as recommended by Simoneau et al. (1986).

3.5.2.2 Capillary Density

Additional serial cross-sections (16μm) of the muscle were stained for capillary density using the periodic acid Schiff-amylase method (Andersen and Henriksson 1977).

3.5.2.3 Enzyme Analyses

Metabolic enzymes measured were the fatty acid β-oxidative enzyme HAD and the glycolytic enzymes PFK and LDH. Muscle samples for analysis of PFK, HAD and LDH were homogenised in a solution containing 170 $mmol.L^{-1}$ KH_2PO_4; 0.02% BSA and 5 $mmol.L^{-1}$ mercaptoethanol adjusted to pH 7.4. The muscle was homogenised on ice using an electric homogeniser (OMNI International S/N TH-1276, Warrenton, USA) in the following manner. A dilution factor of 1:100 wt/Vol was used and the muscle was homogenised with 3 bursts of 15s at a medium speed separated by 15s rest and a fourth burst of 10s at maximal speed. Enzyme analyses were performed immediately post – homogenization for PFK while HAD and LDH analyses were performed on stored homogenates. The activity of PFK was determined on the homogenate using fluorometric methods previously described by Green et al. (1984). LDH was assayed on stored homogenate using a reaction mixture (pH 7.0) containing 100mM Imidazole, 1.0mM pyruvate, 0.04% BSA and 25μM NADH based on the method of Costill et al. (1976). HAD was assayed on stored homogenate with 150mM Imidazole-HCL buffer (pH 6.0), 1mM EDTA, 200μM S-aceto-acetyl CoA, 0.05% BSA and 100μM NADH in the reaction mixture (Chi et al. 1983). A luminescence spectrometer (Aminco Bowman AB2, Urbana, USA) was used to measure the rates of activity of the enzymes measured. Muscle citrate synthase was also determined as discussed in section 3.5.2.4.

3.5.2.4 Mitochondrial ATP Production Rate (MAPR).

Mitochondrial Preparation

Fresh muscle was placed on a petri dish over ice, dissected free of visible fat and connective tissue, minced finely and weighed. The muscle sample was then placed into a 5 mL ground glass

tissue grinding tube (Kontes, 885502-0021, New Jersey) and 1000 μL pre-cooled homogenizing solution (solution A) was added. A ground glass pestle (Kontes 885501-0021) was then used to grind the tissue lightly with a rolling motion until no visible fragment of muscle remained. The resulting solution was transferred to an eppendorf centrifuge tube, which was stored on ice. The pestle and tube were rinsed clean with a further 500 μL of solution A, which was collected and added to the eppendorf tube containing the muscle homogenate. Homogenising solutions for the preparation of isolated mitochondria and the isolation procedures are those originally described by Wibom et al. (1990). The homogenate was then centrifuged in a cold centrifuge (Heraeus Biofuge 28RS) at 650G for 3 minutes at 4°C to separate any connective tissue from the mitochondria. The supernatant was collected and transferred to a new eppendorf tube, which was spun again at 15,000G for 3 minutes at 4°C to cause the mitochondria and other remaining tissue to sediment. The supernatant was discarded and the pellet resuspended with 1000μL of solution A. The ensuing mixture was then centrifuged for a further 3 minutes at 15,000G. Solution A contained Tris (hydroxymethyl) methylamine (50mmol.L^{-1}), KCl (100mmol.L^{-1}), $MgCl_2$ (5mmol.L^{-1}), ATP (1.8mmol.L^{-1}) and EDTA (1mmol.L^{-1}) adjusted to pH 7.2 with HCl.

The supernatant was discarded and the pellet suspended in 200μL of the mitochondrial storage solution (solution B). This was then mixed in the tip of a glass pasteur pipette, ensuring no air bubbles for 2-3 minutes until there were no remaining lumps. 100μL of the suspension was then diluted in a further 400μL of solution B. Solution B contained sucrose (180mmol.L^{-1}), KH_2PO_4 (35mmol.L^{-1}), magnesium acetate (5mmol.L^{-1}) and EDTA (1mmol.L^{-1}), adjusted to pH 7.5 with KOH.

10μL of the 1:5 dilution of the mitochondrial suspension was further diluted with 490μL of the ATP monitoring reagent solution (AMRS). AMRS was prepared by diluting the commercially provided solution (FL-AAM, Sigma) 12.5 fold with solution C. Solution C contained $Na_4P_2O_7$ ($1mmol.L^{-1}$), magnesium acetate ($10mmol.L^{-1}$), BSA (1mg/mL), sucrose ($180mmol.L^{-1}$), KH_2PO_4 ($35mmol.L^{-1}$) and EDTA ($1mmol.L^{-1}$), pH 7.5 with KOH. Overall the original mitochondrial suspension was diluted to a 1:250 mitochondrial suspension solution and was placed on ice ready for MAPR analysis. The remainder of the 1:5 diluted mitochondrial suspension was used for measurement of protein content in the mitochondrial suspension according to the method of Lowry et al. (1951).

MAPR assay

MAPR was determined at 25°C, using the method and substrate concentrations described by Wibom and Hultman (1990) as modified in our laboratory and described in Wang et al. (1999). 10μL aliquots of the 1:250 mitochondrial suspension were added to cuvettes containing highly pure ADP, AMRS (FL-AAM, Sigma) and a range of substrates used to represent fat, protein and carbohydrate metabolism. The substrate combinations utilized were as follows: pyruvate ($1mmol.L^{-1}$) and malate ($1mmol.L^{-1}$) (P&M); palmitoyl carnitine ($0.005mmol.L^{-1}$) and malate ($1mmol.L^{-1}$) (PC&M); alpha-ketoglutarate ($10mmol.L^{-1}$) (α-kg); succinate ($20mmol.L^{-1}$) and rotenone ($0.1mmol.L^{-1}$) (S&R) and pyruvate, palmitoyl carnitine, alpha-ketoglutarate and malate (PPKM). To account for the effect of the adenylate kinase and other non-specific reactions (Wibom et al. 1990) in ATP production, cuvettes containing ADP ($40mmol.L^{-1}$) and mitochondrial suspension (10μL of 1:250 diluted mitochondrial suspension), but no substrates, were run as blanks. MAPR was determined by the rate of light production, which was measured

on a custom designed luminometer comprising a photo multiplier tube attached to a luminescence spectrometer (Aminco Bowman AB2, Urbana, USA). Cuvettes containing the substrates in the six combinations previously described were made up in duplicate. Light emission was measured at 25°C in the luminescence spectrometer chamber following incubation in a 25°C water bath for 5 minutes. Between each measurement the cuvettes continued to be incubated in a water bath at 25°C. After the rate of ATP production had been established 197 pmol ATP standard (FL-AAS, Sigma) in 10μL was added to each cuvette as an internal ATP standard, to allow for calculation of MAPR. MAPR is expressed as $mmol.min^{-1}.kg^{-1}$ muscle and $mmol.min^{-1}.g^{-1}$ mitochondrial protein. MAPR was analysed on fresh muscle and was completed within 4 hours of the biopsy. Mitochondrial suspension proteins were analysed in triplicate according to the method of Lowry (1951).

Citrate Synthase (CS)

CS, a TCA cycle enzyme is specific as a 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 disruption of the mitochondrial membranes with Triton X-100 and 3 freeze thaw cycles. A 50μL aliquot of the original mitochondrial suspension was diluted with 150μL of solution B for the measurement of CS activity in the mitochondrial suspension prior to rupture of the mitochondrial membrane (CSmb). For measurement of CS activity after disruption of the mitochondrial membrane (CSma), a 50μL aliquot of the original mitochondrial suspension was diluted using 150μL of a solution containing 0.1% Triton X-100, $175mmol.L^{-1}$ KCl and $2mmol.L^{-1}$ EDTA adjusted to pH 7.4 with KOH. A separate piece of muscle was used to

determine CS activity in whole muscle (CStotal). The CStotal was homogenised in the same solution used to dilute the CSma sample in a 1:50 dilution (wt/vol). The muscle samples were homogenised using an electrical homogeniser (OMNI International S/N TH-1276, Warrenton, USA) using the method already described for other metabolic enzymes. The CS assay in use in our laboratory is based on that originally described by Srere (1969). The yield of intact mitochondria in the mitochondrial suspension was calculated as the ratio of CS_{im} to CStotal. CS activity and was determined at 25°C with spectrophotometric detection (LKB Biochrom, Ultrospec II) at a wavelength of 412nm. The reaction was followed for 3 minutes and duplicate assays were performed for each of the CSmb, CSma and CStotal samples.

3.5.2.5 Muscle iNOS and nNOS Expression

These methods will be discussed in detail in the methods section of chapter 6.

3.6 ANGIOTENSIN CONVERTING ENZYME (ACE) GENOTYPE

This method will be discussed in detail in the methods section of chapter 7.

3.7 STATISTICAL ANALYSES

Results for two comparisons between subject groups were analysed by independent t-tests (e.g. CHF patients vs. healthy controls, Chapter 4). 2 way analyses of variance (ANOVA) were used to compare differences within and between groups in the longitudinal studies (e.g. Chapter 5, training vs. control patients). A level of significance of $P<0.05$ was accepted for all analyses. All experiment data are presented as mean ± standard error of the mean (SEM) except for population statistics (e.g. age) which are presented as mean ± standard deviation (SD).

CHAPTER 4. SKELETAL MUSCLE METABOLISM AND MORPHOLOGY IN CHRONIC HEART FAILURE

4.1. INTRODUCTION

Compared to healthy subjects, patients with chronic heart failure (CHF) exhibit a reduced exercise tolerance characterised by low peak oxygen consumption ($\dot{V}O_{2\,peak}$) and the early onset of fatigue (Clark et al. 1996) and lactic acidosis (Näveri et al. 1997) during incremental exercise. These symptoms were formerly considered to be due to impaired cardiac function in these patients. There is no correlation, however, between $\dot{V}O_{2\,peak}$ and indices of left ventricular performance (Cohn et al. 1993). Further, in CHF patients the addition of arm exercise to maximal leg exercise produces increases in $\dot{V}O_{2\,peak}$ suggesting that exercise capacity in these subjects is limited by muscle function not cardiac output (Joundeau et al. 1992).

Previously deficits in exercise tolerance similar to those described above for CHF patients have been observed in lung transplant recipients (Wang et al. 1999). 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 fibres 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 fibre type composition and fibre atrophy or loss leading to reduced muscle strength (Massie et al. 1987; Lipkin et al. 1988; Wilson et al. 1993; Sullivan et al. 1997). It is possible that reduced muscle

blood flow is also an important component in exercise impairment in these patients (Wilson et al. 1993).

The effects of CHF on skeletal muscle oxidative capacity are unclear. Whilst a number of reports have suggested reduced activity of oxidative enzymes, this is not universally the case. Sullivan et al. (Sullivan et al. 1990) report reductions in both CS and SDH activity in CHF patients while (Opasich et al. 1996) 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 (Duscha et al. 1999; Duscha et al. 2001; Schaufelberger et al. 2001). Additionally no difference in *in vitro* oxygen consumption in intact mitochondria has been noted between CHF patients and sedentary healthy control subjects despite significant differences between the groups in exercise tolerance (Mettauer et al. 2001). Interestingly, studies examining the effects of CHF on oxidative phosphorylation *in vivo* have consistently reported reduced rates of oxidative phosphorylation in CHF patients (Massie et al. 1988; Mancini et al. 1992). 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 myocytes (Wibom and Hultman 1990). However, no previous studies have examined MAPR in CHF patients.

The aim of the present study was to determine the role of skeletal muscle phenotypical (histochemical and metabolic) maladaptations to CHF. In particular, it was 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 fibres, muscle fibre atrophy and lower capillary density.

4.2. METHODS

4.2.1. Subjects.

Seventeen stable chronic heart failure (CHF) patients (16 male/1 female; 68 ± 8 years, 84 ± 16 kg, body mass index 28 ± 5 kg/m^2; mean ± S.D.) and eight healthy sedentary control subjects (7 male/1 female; 63 ± 11 years, 78 ± 8 kg, body mass index 26 ± 3 kg/m^2) participated in this study. The CHF group were in New York Heart Association functional class (NYHA) II (n = 13) & III (n = 4) and had left ventricular ejection fractions (LVEF) of 27 ± 8%. The average time since diagnosis of CHF in the patients was 39 ± 41 months. Two of the 17 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.

Table 4.1. Descriptive characteristics of the 17 CHF patients

	CHF patients
CHF diagnosis	
Ischemic heart disease	11 (65%)
Dilated cardiomyopathy	5 (29%)
Valvular	1 (6%)
Medications	
Angiotensin converting enzyme inhibitor	14 (82%)
Angiotensin receptor blocker	3 (18%)
Diuretic	15 (88%)
Beta-blocker	8 (47%)
Digoxin	7 (41%)
Aspirin	13 (76%)
Warfarin	5 (29%)
Amiodarone	2 (12%)
Nitrates	7 (41%)
Calcium channel antagonist	2 (12%)

4.2.2. Incremental Exercise Tests and blood sampling.

Peak total body oxygen consumption ($\dot{V}O_{2\,peak}$) was determined on CHF patients and healthy subjects as described in section 3.2.1. Arterialised venous blood samples were obtained as previously described. Oxygen saturation was consistently in excess of 95% as confirmed by pulse oximetry and blood gas analysis, confirming arterialisation.

4.2.3. Plasma Lactate, Calculation of Lactate Threshold and Predicted $\dot{V}O_{2\ peak}$.

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. work rate (W) as described by Beaver et al. (1985). Predicted $\dot{V}O_{2\ peak}$ was calculated according to the formulae of Wasserman et al. (1994).

4.2.4. Muscle Strength Testing.

Unilateral (right leg) skeletal muscle strength for knee extension / flexion was assessed as previously described (section 3.2.2).

4.2.5. Body Composition.

Fourteen of the CHF patients and the eight healthy control subjects underwent total body and thigh DEXA scanning to determine total body and lean thigh mass, as previously described.

4.2.6. 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. Biopsy samples were used to determine MAPR, muscle enzymes, fibre type, fibre area and capillary density as previously described (Chapter 3). The enzymes CS, HAD, PFK and LDH were selected due to their activities in key metabolic pathways. CS catalyses the formation of citric acid from oxaloacetate and acetyl coenzyme A in the initial step in the Krebs' cycle and consequently can be considered a marker enzyme for oxidative metabolism. HAD is a rate limiting enzyme in β-oxidation and its activity can

therefore give an indication of the potential ability to recruit fatty acids for energy within the cell. PFK is a rate limiting enzyme in glycolysis and consequently its activity will give an indication of rate of flux through this metabolic pathway. LDH catalyses the formation of lactic acid from pyruvate. This reaction is increased during periods when metabolic demand exceeds the capacity of oxidative metabolism to resynthesise ATP. Consequently elevated LDH activities will reflect periods of high metabolic stress.

Fibre type percentages were determined from sections containing 204 ± 19 (mean ± S.E.) fibres. Entire sections stained for capillaries were used to compute capillary to fibre ratio (203 ± 13). Mean capillary contacts per fibre type were determined on 20 contiguous fibres of each type except where there were insufficient fibres of a particular type. In this case capillary contacts for the fibre type were made on the number of fibres of that type available.

4.2.7. Statistical Analysis.

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

4.3. RESULTS

4.3.1. Exercise Tolerance.

Compared with their healthy counterparts CHF patients had significantly lower peak oxygen consumption ($\dot{V}O_{2\,peak}$; 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 4.2). The CHF patient group had an earlier lactate threshold (Table 4.3), 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 4.3). 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 4.3). No patient exhibited arterial oxygen desaturation at the termination of exercise.

Table 4.2. Skeletal muscle strength and $\dot{V}O_2$ peak data for CHF Patients and Healthy Subjects

	CHF	**Healthy**
$\dot{V}O_2$ peak ($ml.kg^{-1}.min^{-1}$)	15.0 ± 0.9	28.1 ± 2.3‡
% Predicted $\dot{V}O_2$ peak	64 ± 4	111 ± 9‡
Knee Extension (Nm)	109 ± 10	133 ± 6*
Knee Flexion (Nm)	46 ± 4	64 ± 4†

Values are mean ± SEM. *Denotes $p < 0.05$. †Denotes $p < 0.01$. ‡Denotes $p < 0.001$.

Table 4.3. Plasma Lactate and Workload Data for CHF Patients and Healthy Subjects

	CHF	**Healthy**
Peak Watts	68 ± 7	138 ± 13‡
Lactate at fatigue (mmol/L)	4.7 ± 0.5	8.2 ± 0.9†
Lactate Threshold (W)	30.6 ± 3.5	81.6 ± 8.6‡
Lactate Threshold/ Peak Watts (%)	48.6 ± 2.7	61.7 ± 3.3†
Peak Respiratory Exchange Ratio (RER)	1.15 ± 0.03	1.16 ± 0.03

Values are mean ± SEM. †Denotes $p < 0.01$. ‡Denotes $p < 0.001$.

4.3.2. 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 4.1). 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 enzymes PFK and LDH (Table 4.4).

Table 4.4. Skeletal Muscle Enzyme Activity in CHF patients and Healthy Subjects

Enzyme	CHF	Healthy
Citrate Synthase	14.05 ± 0.87	14.63 ± 0.85
β-Hydroxyacyl-CoA Dehydrogenase	12.51 ± 0.89	13.58 ± 1.25
Phosphofructokinase	28.76 ± 1.29	27.47 ± 1.13
Lactate Dehydrogenase	59.11 ± 7.38	79.75 ± 13.69

Values are mean ± SEM, expressed as μmol/min/g wet weight of muscle.

4.3.3. Muscle Fibre Types.

Histochemical data was obtained from 16 CHF patients and seven controls. CHF patients exhibited a lower proportion of type IIA muscle fibres (20.4 ± 2.1 vs. 35.3 ± 1.9; $p < 0.0001$) and higher proportion of type IIX muscle fibres (32.6 ± 1.8 vs. 18.5 ± 1.7; $p < 0.0001$) than the healthy subjects (Figure 4.2). No difference was found in the proportion of type I fibres ($p = 0.65$) between the CHF and healthy subjects (Figure 4.2). However, type I fibre cross-sectional area (μm^2) was lower in the CHF patient group than the healthy subjects (3085 ± 276 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 fibres (Table 4.5).

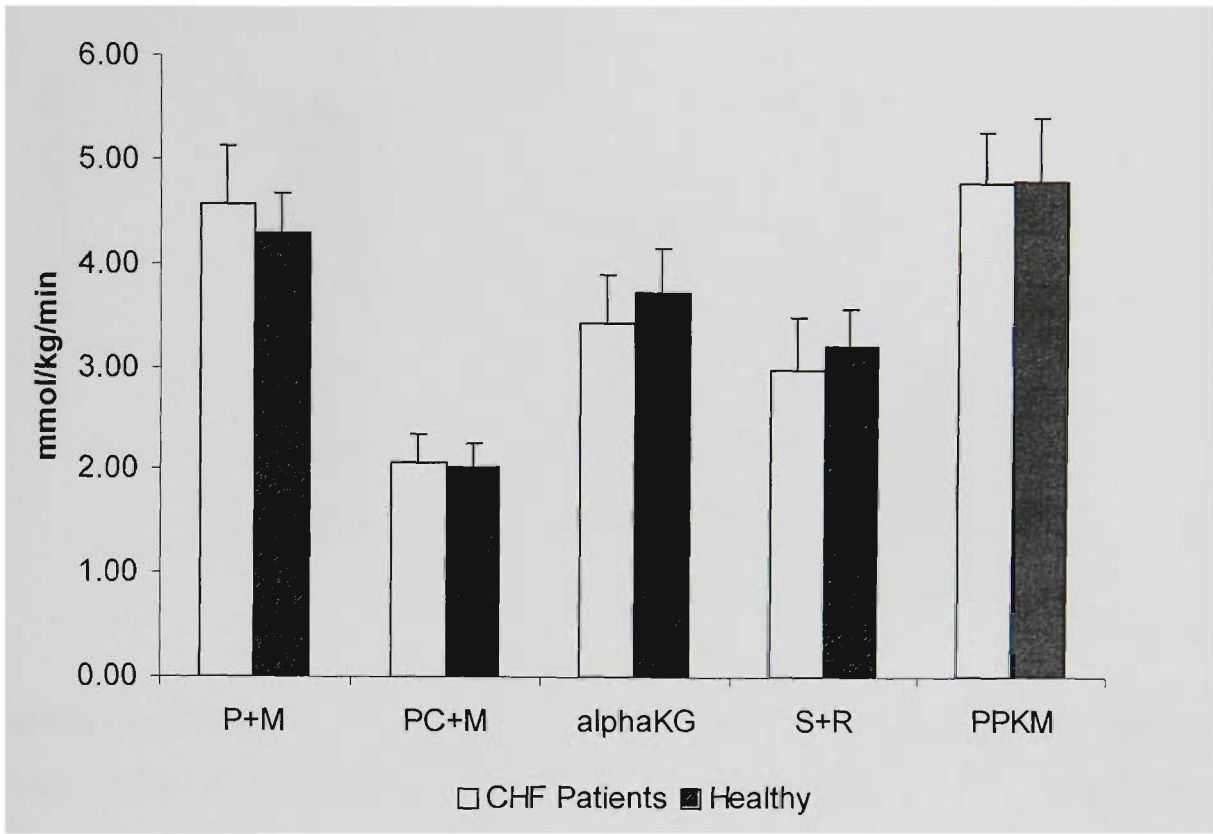


Figure 4.1. Mitochondrial ATP Production Rate in Patients With Class II-III CHF and Healthy Subjects (Mean ± SEM). The substrate combinations were: P+M = pyruvate + malate; PC+M = palmitoyl-L-carnitine + malate; α-KG = α-ketoglutarate; S+R = succinate + rotenone; PPKM = pyruvate + palmitoyl-L-carnitine + α-ketoglutarate + malate. Values are mean ± SEM.

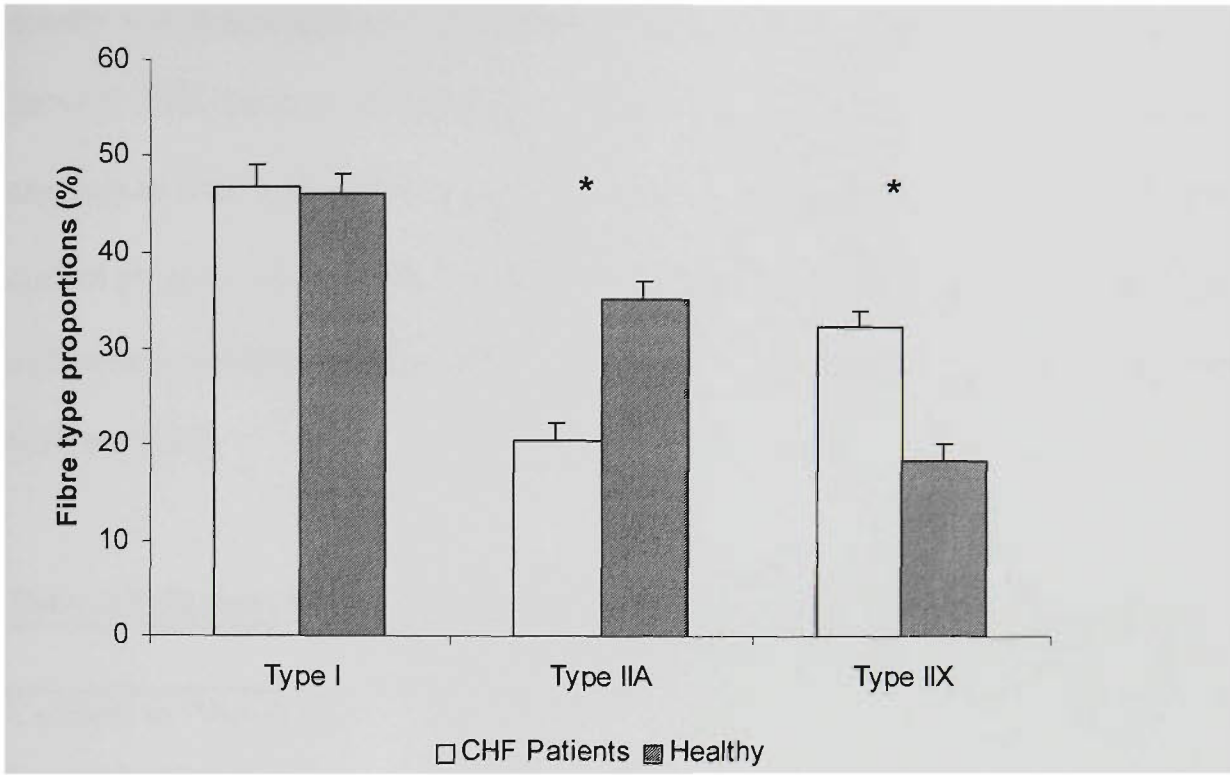


Figure 4.2. Skeletal Muscle Fibre Type Proportions in 16 CHF Patients and 7 Healthy Subjects. Mean number of fibres used to compute fibre type proportions = 190 ± 16 Values are mean ± SEM. # denotes $p < 0.0001$.

Table 4.5. Muscle Fibre areas.

Fibre Areas (μm^2)	**CHF (n=16)**	**Healthy (n=7)**	**P**
Type I	3085 ± 276	3840 ± 270	0.048
Type IIA	2958 ± 248	2934 ± 195	0.932
Type IIX	2392 ± 196	2430 ± 170	0.866

Data is presented as Mean ± SEM

4.3.4. Capillary Density.

Capillary to fibre ratio was lower in CHF patients ($p < 0.001$) than in control subjects (Table 4.6). CHF patients exhibited fewer capillaries surrounding type I fibres ($p < 0.001$) and type IIX fibres ($p < 0.05$) than controls. A trend was observed for fewer capillaries surrounding type IIA fibres ($p = 0.075$) in CHF patients compared to healthy controls. However, when capillary

density was determined per unit of muscle fibre cross-sectional area, there was no difference between CHF patients and control subjects (Table 4.6). There was no correlation between capillary to fibre ratio and $\dot{V}O_{2\,peak}$ 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 fibre ratio and muscle strength in either the CHF patients ($r = 0.008$, $p = 0.98$) or healthy subjects ($r = 0.34$, $p = 0.50$).

Table 4.6. Skeletal Muscle Capillary Density in CHF Patients and Healthy Subjects

	CHF (n=16)	**Healthy (n=7)**
Capillary to Fibre Ratio	1.11 ± 0.05	1.40 ± 0.04‡
Capillaries Contacting Individual Fibres		
Type I	3.08 ± 0.11	3.82 ± 0.11†
Type IIA	2.78 ± 0.13	3.06 ± 0.10
Type IIB	2.22 ± 0.10	2.46 ± 0.08
Capillaries per mm^2 of Fibre Area		
Type I	1123 ± 76	1122 ± 87
Type IIA	1042 ± 40	1082 ± 65
Type IIB	1060 ± 52	1118 ± 100

Data presented as mean ± SEM. Mean number of fibres used to compute capillary to fibre ratio = 200 ± 13. *Denotes $p < 0.05$. †Denotes $p < 0.001$. ‡Denotes $p < 0.001$.

4.3.5. Body Composition and Lean Thigh Mass.

No difference was found between the CHF patients and the normal controls in either lean body mass or lean thigh mass (Table 4.7). There was a significant correlation between thigh lean mass and $\dot{V}O_{2\,peak}$ for the CHF patients but this did not reach statistical significance in the healthy

subjects (Figure 4.3). Additionally whole body lean mass was also significantly correlated with $\dot{V}O_{2\,peak}$ ($r = 0.713$; $p<0.05$) in CHF patients but again this did not reach significance in healthy subjects.

Table 4.7. Total Body and Thigh Lean Masses and Lean Thigh/Total Body Mass Ratio

	CHF (n = 14)	**Healthy**
Lean Thigh Mass (kg)	5.29 ± 0.26	5.87 ± 0.31
Lean Body Mass (kg)	50.67 ± 1.88	52.83 ± 2.43
Lean Thigh/Body Mass %	6.89 ± 0.20	8.01 ± 0.34*

Data presented as mean ± SEM. *Denotes $p < 0.05$.

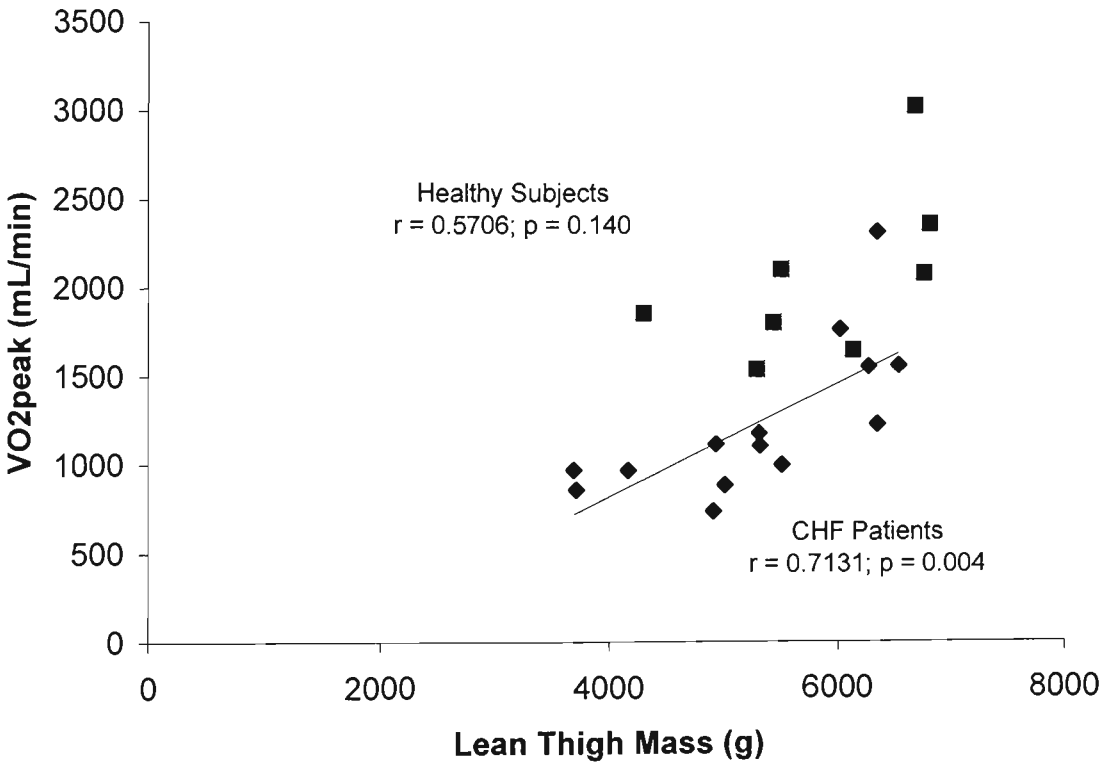


Figure 4.3. Correlations between Lean Thigh Mass and $\dot{V}O_{2\,peak}$ in Healthy Subjects (squares) and CHF Patients (diamonds)

4.4. DISCUSSION

4.4.1. Main Findings in this Study.

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 $\dot{V}O_{2\,peak}$, there is no difference in MAPR between CHF patients and similarly aged healthy individuals. This result is consistent with the lack of difference in mitochondrial oxygen consumption, measured using in situ mitochondria in saponin-skinned skeletal muscle fibres, between CHF patients and sedentary control subjects that has been reported previously (Mettauer et al. 2001). Significantly however, Mettauer et al. (2001) reported lower citrate synthase (CS) activity in the CHF patients compared to sedentary controls suggesting that mitochondrial enzyme activities are not necessarily the best indicators of oxidative capacity. Other 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 CS (Sullivan et al. 1990; Mettauer et al. 2001; Schaufelberger et al. 2001), HAD (Duscha et al. 1999; Duscha et al. 2001) and SDH (Sullivan et al. 1997; Duscha et al. 1999) have been reported. Other studies have reported no differences in CS (Opasich et al. 1996; Sullivan et al. 1997; Duscha et al. 1999; Duscha et al. 2001), HAD (Schaufelberger et al. 2001) and SDH (Opasich et al. 1996). Nevertheless nuclear magnetic resonance (NMR) studies of muscle metabolism in patients affected by CHF have found more rapid depletion of phosphocreatine (PCr) during exercise (Massie et al. 1988; Mancini et al. 1992) and lower rates of post-exercise PCr resynthesis (Mancini et al. 1992) 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 function (Letellier et al. 1994) making their measurement a less than ideal way of determining maximal oxidative capacity. 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 (Wibom and Hultman 1990; Wibom et al. 1992; Starritt et al. 1999). Previous studies have demonstrated that MAPR is strongly associated with $\dot{V}O_{2\,peak}$. It is higher in endurance trained individuals compared to sedentary subjects (Wibom and Hultman 1990) and increases with endurance training (Wibom et al. 1992; Starritt et al. 1999). 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 no difference was found between the groups in the activity of the mitochondrial enzymes CS and HAD. Clearly there is a lack of consistency in the literature and 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. They were not selected because of cachexia or severe functional impairment nor had they been specifically referred for cardiac transplantation. This is reflected in the mean age 68 ± 9 years with a mean LVEF 27 ± 8% on gated blood pool

scanning. Other studies may have included patients with cachexia and / or greater levels of functional impairment, which may in part explain the differences between the current results and those of previous studies. The observations reported here demonstrate that the reduced maximal oxygen consumption in this cohort of CHF patients must be at least partly due to factors other than muscle oxidative capacity.

4.4.2. Muscle Fibre Types.

Muscle biopsy studies in CHF have consistently demonstrated changes in muscle histochemistry including a reduced capillary to fibre ratio (Duscha et al. 1999) and an increase in type IIX fibre proportions at the expense of either type I (Sullivan et al. 1990; Massie et al. 1996) and/or type IIA muscle fibres (Duscha et al. 1999; Schaufelberger et al. 2001). Significant muscle fibre atrophy (Massie et al. 1996) and reduced total muscle cross sectional areas (Mancini et al. 1992) have also been reported in CHF patients. Alterations in fibre type proportions are a potential cause of the exercise intolerance in CHF. The proportion of type IIA muscle fibres was lower and the proportion of type IIX fibres wa s higher in the CHF group in the current study. Paradoxically, the increased proportion of type IIX muscle fibres did not result in a lower muscle oxidative capacity in CHF. Bigard et al. (1998) however have demonstrated that muscle deconditioning induced by hindlimb suspension in rats results in slow to fast phenotype transitions without change in mitochondrial function. Consequently fibre type transformations do not necessarily result in reductions in overall muscle oxidative capacity.

4.4.3. Capillary Density.

The major function of the capillary bed in skeletal muscle is to supply oxygen and nutrients to the muscle fibres. Endurance training stimulates an increase in capillary density in human subjects without heart failure (Coggan et al. 1992b; Kiens et al. 1993) indicating a potential relationship between capillary density and $\dot{V}O_2$ peak. In the present study, however, there was no correlation between $\dot{V}O_2$ peak and the capillary to muscle fibre ratio in the CHF group. This observation is supported by the findings of Duscha et al. (1999) who found no significant difference in capillary to fibre ratio between the most exercise-impaired CHF patients (< 4 METS) and the normal control group.

In the current study the capillary to fibre ratio and capillary contacts per fibre were lower in the CHF group in comparison to the healthy subjects. When capillary density was calculated relative to fibre area (capillaries/mm^2) however, no difference between the CHF patients and the healthy control subjects was observed. This highlights the importance of measuring capillary density as a function of muscle fibre size and indicates that capillary density is not a factor in the reduced exercise tolerance in CHF patients. This finding is also supported by DeSousa et al. (2000) who reported no difference in capillaries relative to fibre area between CHF and sham-operated rats in either oxidative (soleus) or glycolytic (gastrocnemius) muscles.

4.4.4. Role of Muscle Mass in Reduced Exercise Capacity.

$\dot{V}O_{2\,peak}$ (ml/min) was significantly correlated with lean thigh mass in the CHF patients but less so in the normal healthy controls (Figure 4.3). This relationship in CHF patients has previously been observed in larger patient groups (Harrington et al. 1997; Anker et al. 1999a) suggesting

that reduced muscle mass could be a determinant of the reduced exercise capacity in CHF. Although the mean lean thigh and lean body mass in the current study 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 fibres, a lower lean thigh mass to body mass ratio and reduced thigh muscle strength (Table 4.2). Previous studies using computerized tomography (Harrington et al. 1997) and magnetic resonance imaging (Mancini et al. 1992; Minotti et al. 1993) have demonstrated smaller muscle cross sectional areas in CHF patients. 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 $\dot{V}O_{2\,peak}$ in the CHF patients compared to the healthy control subjects.

4.4.5. Metabolic Control.

While the current data demonstrate that skeletal muscle oxidative capacity is not reduced in the CHF patients recruited for this study, 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 PCr resynthesis post-exercise in CHF patients (Mancini et al. 1992; Cohen-Solal et al. 1995). As PCr resynthesis is an oxidative process (Kemp et al. 1993), this suggests an impaired rate of oxidative phosphorylation. Cohen-Solal et al. (1995) 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 (Massie et al. 1987; Okita et al. 1998). 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. The earlier onset of lactic acidosis may be related to the reduction in the cross-sectional area of the type I fibres leading to the recruitment of type II muscle fibres at lower exercise intensities. Additionally, since the CHF group had a significantly lower proportion of type IIA fibres an earlier reliance on glycolytic type IIX fibres during the incremental exercise test would be expected.

Other factors may also be responsible for reducing oxidative phosphorylation during exercise in CHF. Toussaint et al. (1998) have shown reduced reactive hyperaemic 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.

Additionally, 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, fibre type alterations that increase glycolytic fibre proportions would reduce the sensitivity of whole muscle oxidative phosphorylation to ADP stimulation. The higher proportion of glycolytic fibres in CHF patients than in controls may explain the aforementioned lower levels of oxidative phosphorylation during exercise. (DeSousa et al. 2000) A further mechanism related to the control of oxidative phosphorylation during exercise, as described by Conley et al.(2001), 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

$$PCr + ADP + H^{+} \leftrightarrow ATP + Cr$$

The ADP level is the central signal for oxidative phosphorylation. Conley et al. (2001) point out that the rise in ADP as PCr levels fall is attenuated at lower intramuscular pH. In the current CHF group higher proportions of type IIX glycolytic muscle fibres and an earlier onset of lactic acidosis in incremental exercise were observed, potentially reflecting a lower muscle pH. It is postulated, 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. (1987) have observed a lower muscle pH during submaximal exercise in CHF patients compared to healthy control subjects.

4.5 CONCLUSION.

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 appear to have normal resting skeletal muscle oxidative function. The earlier onset of lactic acidosis however is consistent with reduced oxidative function during exercise.

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

Lower lean body mass and type I fibre atrophy are both associated with the impairment in $\dot{V}O_{2\,peak}$. Potentially these morphological changes in skeletal muscle might be reversible following muscle-strengthening exercise training and / or better treatment of the underlying heart failure.

CHAPTER 5. THE EFFECT OF RESISTANCE TRAINING ON SKELETAL MUSCLE IN CHRONIC HEART FAILURE

5.1. INTRODUCTION

Chronic heart failure (CHF) is characterized by a low peak oxygen uptake ($\dot{V}O_{2\ peak}$) that is a stronger predictor of morbidity and mortality (Bittner et al. 1993) than the level of impairment of cardiac performance (Franciosa et al. 1981; Cohn et al. 1993). The low $\dot{V}O_{2\ peak}$ is associated with impaired exercise tolerance and early onset of lactic acidosis in incremental exercise (chapter 4). Muscle oxidative function is reduced in CHF and it is widely accepted that this is the cause of exercise intolerance in this patient group rather than impaired cardiac function. Additionally several studies have reported significant muscle atrophy in CHF patients which may also contribute to the reduced exercise capacity. These patients also exhibit reduced muscle strength due, at least in part, to the significant muscle atrophy (Harrington et al. 1997). Both low exercise capacity and strength contribute to reduced quality of life in these patients and exercise rehabilitation needs to address both of these areas of impaired function.

Traditionally endurance training (ET) has been the recommended modality to increase $\dot{V}O_{2\ peak}$ and muscle oxidative capacity however this form of training has little influence on muscle mass (Meredith et al. 1989; Sipila and Suominen 1995). Conversely resistance training (RT) results in increases in muscle strength and skeletal muscle hypertrophy in healthy young subjects (Higbie et al. 1996; McCall et al. 1996) with no change in muscle oxidative capacity (Wang et al. 1993) or capillary density (McCall et al. 1996). Phenotypical changes that occur in response to RT in young subjects include a transition from type IIX to IIA fibres (Wang et al. 1993). Likewise, studies which have investigated the effects of RT in healthy elderly subjects have reported

significant gains in muscle strength and associated muscle hypertrophy (Frontera et al. 1990; Hikida et al. 2000) and fibre type transitions from IIX to IIA fibres (Hikida et al. 2000). However, in elderly subjects RT has also been demonstrated to increase endurance exercise capacity ($\dot{V}O_{2\ peak}$) in conjunction with increases in skeletal muscle oxidative capacity (Frontera et al. 1990) and in the capillary to fibre ratio (Hepple et al. 1997). Additionally, Hepple et al. (1997) reported an increase in the capillary to fibre perimeter exchange index which paralleled their reported changes in. There is also evidence that RT in elderly subjects results not only in muscle hypertrophy but also increased $\dot{V}O_{2\ peak}$ (Frontera et al. 1990).

Three studies have investigated the effect of a RT program in CHF patients. One of these studies (Selig et al. 2003) comprised a group of CHF patients (n = 38) of which a subgroup of volunteers consented to a skeletal muscle biopsy. The muscle biopsy group form the major focus of the present study but exercise tolerance data from the complete cohort in the study reported by Selig et al. (2003) is referred to in this chapter. A previous study by Hare et al. (1999) reported an increase in submaximal exercise tolerance and muscle strength, but no improvement in $\dot{V}O_{2\ peak}$ following 11 weeks of RT. Pu et al. (2001) examined the effect of RT on skeletal muscle characteristics in elderly female CHF patients. They reported trends towards an increase in both the cross-sectional area of the type I fibres and muscle oxidative capacity as indicated by citrate synthase (CS) activity. These changes would be expected to lead to improvements in $\dot{V}O_{2\ peak}$ although such a change was not observed in that study. Both of these studies highlight the potential benefits of RT in this subject group. It has previously been demonstrated however (Duscha et al. 2001; Duscha et al. 2002) that there are differences in skeletal muscle characteristics between men and women with CHF. This may lead to gender specific responses

to RT as demonstrated in endurance training (Keteyian et al. 2003). Thus it is important that trials involving male CHF patients are also conducted. Additionally, the method of MAPR is a direct measurement of mitochondrial ATP production rate in vitro across the entire range of metabolic energy producing pathways and as such is a better indicator of muscle oxidative capacity than the Krebs cycle enzyme CS. The current study examines the effect of RT in CHF on MAPR and also examines the effect of RT on capillary density and body composition. Previous studies examining the effect of RT in CHF have not looked at these factors.

In the present study CHF patients who agreed to a muscle biopsy were randomly allocated to 11 weeks of RT (n = 7) or 11 weeks of normal care (n = 6). The aim was to examine the effects of RT on $\dot{V}O_{2\ peak}$, muscle strength and characteristics of skeletal muscle. It was hypothesized that RT in these patients would be well-tolerated and would result in improvements in muscular strength and $\dot{V}O_{2\ peak}$ due to phenotypical and metabolic adaptations in the skeletal muscle.

5.2. METHODS

5.2.1. Subjects.

Thirteen stable chronic heart failure (CHF) patients (12 male/1 female; 70 ± 8 years, 84 ± 18 kg, body mass index 29 ± 6 kg/m^2; mean ± SD) who were part of the larger cohort and who volunteered for muscle biopsies participated in this study. These subjects were in NYHA functional class II (n = 9) & III (n = 4) and had LVEF of 26 ± 8%. Following familiarisation and baseline testing the patients were randomly allocated to resistance training (Ex: n = 7) or inactive control (C: n = 6) group according to a generated random number method held in sealed envelopes by a third party (Peto et al. 1976). Baseline data of these subjects is presented in

Table 5.1. As previously indicated the subjects and data reported in this chapter form a subgroup of a larger study of resistance training in CHF (Selig et al. 2003). Baseline characteristics of the complete cohort from which this subgroup of volunteers was derived are presented in Appendix II. Strength and $\dot{V}O_{2\ peak}$ data for the entire subject cohort have been included in this chapter due to their relevance to the overall findings. The subgroup of patients that form the main focus of this chapter were the CHF patients who elected to undergo muscle biopsies at baseline and endpoint. 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.

5.2.2. Resistance Training Protocol.

Resistance training involved an 11 week controlled protocol using a hydraulic resistance system that was detailed in section 3.3. Subjects randomised to the control group were encouraged to maintain but not increase normal activity levels.

5.2.3. Incremental Exercise Tests and Blood Sampling.

Details of the incremental exercise testing protocol, respiratory gas and blood analyses were reported in chapter 3.

5.2.4. Calculation of Lactate Threshold.

Plasma lactate levels were determined using Roche Diagnostic Kit reagents as described in chapter 3. Lactate threshold was calculated from a log-log transformation plot of plasma lactate concentration vs. power output (W) (Beaver 1985).

Table 5.1: Characteristics of the biopsy subgroup of CHF patients (mean ± SD)

Characteristic	RT group (n = 7)	Control group (n = 6)	P value
Age, yr	67 ± 9	74 ± 4	0.09
Weight (kg)	88 ± 19	80 ± 18	0.44
Body Mass Index (kg/m^2)	30 ± 6	27 ± 5	0.32
LVEF %	26 ± 8	27 ± 9	0.82
NYHA functional class	2.4 ± 0.5	2.2 ± 0.4	0.34
Etiology			
Ischaemic heart disease	4 (57%)	4 (67%)	
Dilated cardiomyopathy	3 (43%)	1 (17%)	
Valvular	0	1 (17%)	
Medications (Pre/Post)			
Angiotensin converting enzyme inhibitor	6/5	4/4	
Angiotensin receptor blocker	1/2	2/2	
Diuretic	6/6	6/6	
Beta-blocker	5/5	2/3	
Digoxin	4/4	3/3	
Aspirin	4/4	5/4	
Warfarin	3/2	2/3	
Amiodarone	0/2	2/2	
Nitrates	2/2	2/3	
Calcium channel antagonist	1/1	0/0	

5.2.5. 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 (Hare et al. 1999).

5.2.6. Body Composition.

11 CHF patients (5 RT / 6 C) volunteered to undergo DEXA scanning (< 10 microsieverts) to determine body composition and lean tissue mass.

5.2.7. Muscle Biopsy.

Approximately 7d after the baseline and endpoint incremental exercise tests resting muscle biopsies were obtained from the vastus lateralis. This process was detailed earlier (chapter 3). The muscle biopsy sample was used to determine MAPR, metabolic enzymes, muscle fibre type proportions and areas and capillary density.

5.2.12. Statistical Analysis.

Data was compared using two way repeated measures analysis of variance (ANOVA) with group and time as the factors using SPSS statistical analysis software (SPSS v 11.0; Chicago, Illinois). Where differences were identified during ANOVA analysis they were isolated via the use of t-tests. Multiple regression analyses of muscle variables and exercise tolerance were also conducted. Data are expressed as means ± SEM. A p value of less than 0.05 was considered significant.

5.3. RESULTS

5.3.1. Subject Characteristics.

The CHF patients, randomised into control (C) and resistance training (Ex) groups were well matched at baseline in the full patient cohort and in the subgroup of patients who underwent muscle biopsy procedures (summarised in Table 5.1). While there was a tendency for those randomized to the control group to be slightly older than the training subjects ($p = 0.09$), measures of disease severity, pathology and modes of pharmacological control were similar.

5.3.2. Training Compliance and Medication Status.

All of the subjects in the RT group completed a minimum of 33 training sessions. Minimal changes in medications occurred among patients in either group during or in the month prior to commencement of the study (Table 5.1).

5.3.3. Exercise Tests.

There were no significant differences at baseline in either $\dot{V}O_{2\ peak}$, lactate threshold or muscle strength between groups. Significantly different responses in $\dot{V}O_{2\ peak}$ were found between the groups with an increase in the Ex group and decrease in the C subjects (Table 5.2; $p<0.01$) over the course of the 11 week intervention. This occurred in the full cohort (Appendix II) and in the subgroup who underwent muscle biopsies.

There was no significant difference between the groups regarding the work rate (W) at which lactate threshold occurred at baseline (Table 5.2). Lactate threshold (W) increased significantly

in the Ex group (31 ± 3 vs 42 ± 2 W; $p<0.01$) but remained unchanged in C (29 ± 2 vs 30 ± 2 W) following the intervention (Table 5.2).

In the full cohort of patients, those randomised to RT increased their training volumes from an average of 148 ± 27 to 408 ± 37 arbitrary units ($p<0.001$; Fig 5.1). In these patients, strength increased in both the quadriceps ($p<0.05$) and hamstrings ($p<0.05$) muscle groups in the trainers but not the controls following the intervention period (Table 5.2). While significant group by time differences existed in quadriceps muscle strength ($p < 0.05$) only a trend was observed in hamstring muscle strength ($p = 0.09$). In the biopsy subgroup, there were trends towards increases in both quadriceps and hamstrings strength (Table 5.2). Despite the difference in the mean strength of the groups at baseline, subjects randomized to the control group were not significantly stronger than their counterparts in the training group at baseline measurements (p values of .42 & .47 for quadriceps and hamstrings muscle groups respectively).

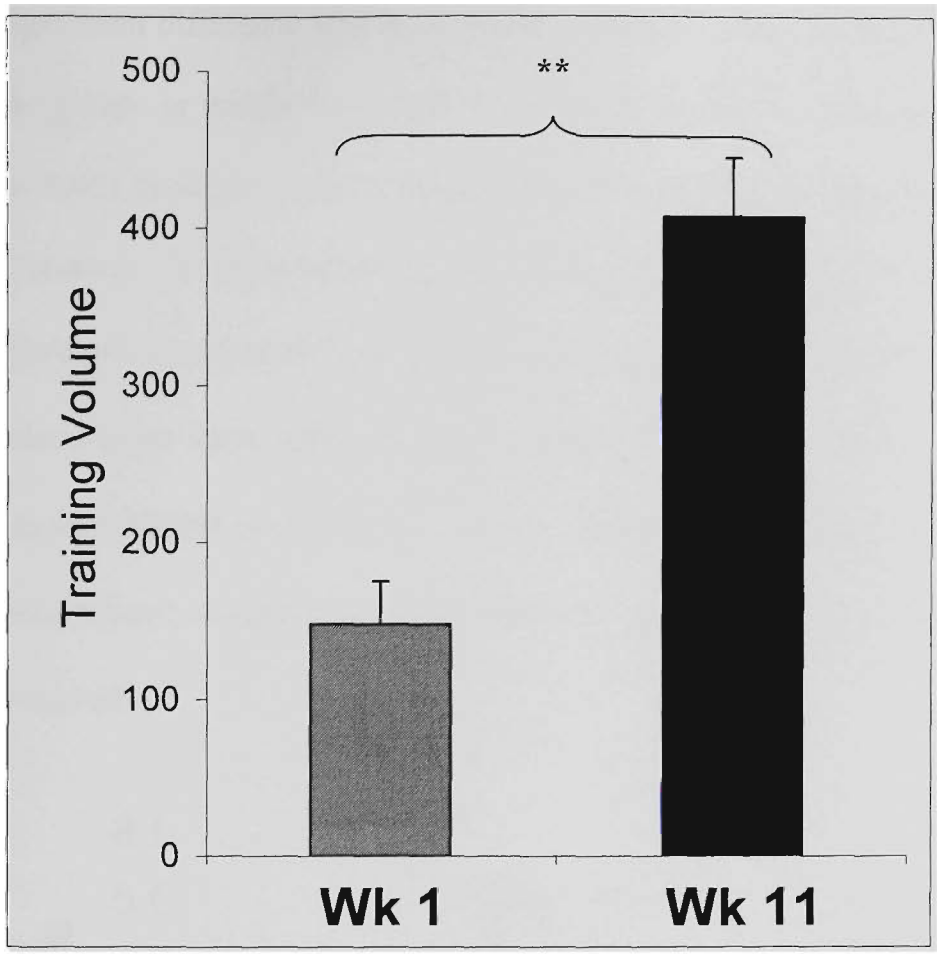


Figure 5.1. Training volumes for patients at the commencement (Wk 1, left bar) and the conclusion (Wk 11, right bar). Volume (arbitrary units) was estimated as the sum of the product of resistance (machine settings) and number of repetitions for each of the exercises. Data are presented as mean ± SD. ** $P < 0.001$.

The respiratory exchange ratio (RER) at fatigue in the incremental exercise test was in excess of 1.1 in both the training and the control groups at baseline and endpoint indicating subjects were similarly motivated throughout.

5.3.4. Muscle Oxidative Capacity.

Using the substrate combination P+M, MAPR increased in the training group ($p<0.05$) and decreased in the control group ($p<0.05$) (Table 5.3) during the 11 week intervention. No

significant difference was found in the response to any of the other MAPR substrates between the groups or within the groups (Table 5.3). Activities of the enzymes CS ($p<0.01$) and HAD ($p<0.05$) increased in the training group (Table 5.3) but were unchanged in the control group following the intervention period. There was no change in the activities of either of the glycolytic enzymes PFK or LDH following the intervention period in either group. Significant relationships were noted between changes in $\dot{V}O_{2\,peak}$ and the indices of muscle oxidative capacity MAPR (P+M: Figure 5.2; $p < 0.0001$) and the oxidative enzyme HAD ($p < 0.05$). A trend towards a significant relationship between changes in CS and $\dot{V}O_{2\,peak}$ ($p = 0.12$) was also observed.

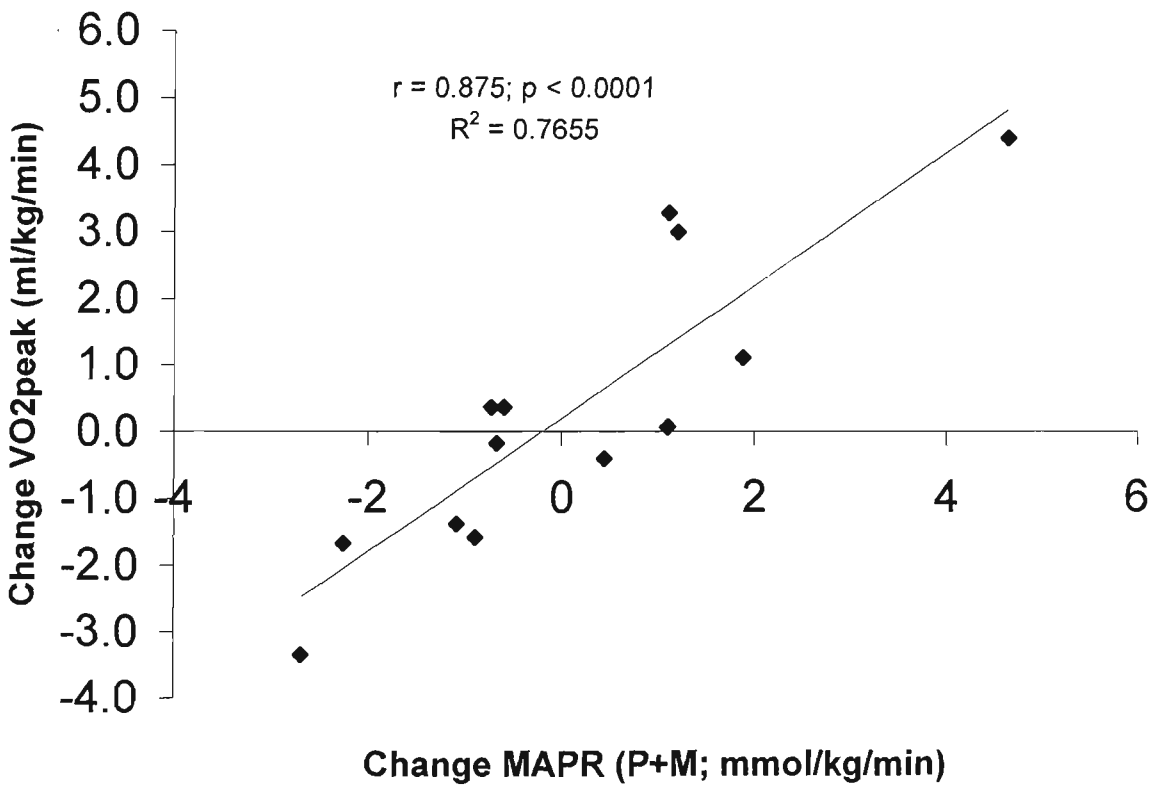


Figure 5.2. The relationship between the change in $\dot{V}O_{2\,peak}$ and skeletal muscle oxidative capacity over the 11 week intervention.

5.3.5. Body Composition.

There was no change in either total lean body mass or lean thigh mass in either of the groups following the intervention (Table 5.4).

5.3.6. Fibre Type Distribution and Mean Fibre Area.

No significant differences were found either between or within the groups in the proportions of any of the three fibre types. There was a trend towards a decrease in the proportion of type I fibres in the control group following the intervention period (Table 5.5; $p = 0.12$). No significant differences were found in fibre area changes as a result of the intervention used. However significant increases were found in the type IIX fibre area of the control group ($p < 0.05$) (Table 5.5).

5.3.7. Capillary Density.

There was a strong tendency for a higher capillary to fibre ratio at baseline in the subjects randomized to the control group (Table 5.6; $p = 0.058$; 2 tailed test). Capillary to fibre ratio increased in the training group following the training intervention (Table 5.6; 1.01 ± 0.05 vs 1.17 vs 0.03; $p < 0.05$) and unchanged (1.15 ± 0.03 vs 1.14 vs 0.03) in the control group. The number of capillaries surrounding oxidative type I fibres increased following the intervention in the RT group (Table 5.6; $p<0.01$) and did not change in the C group although when the changes between the groups over the intervention period were compared there was a trend ($p = 0.076$) for a different response between the groups. There was a trend towards group x time differences in the capillaries surrounding type IIA fibres ($p = 0.066$) with an increase in the mean number of capillary contacts in the RT group and a decrease in the C group. Only the decrease in the

number of capillary contacts on IIA fibres in the control group was significant when compared to baseline values (2 tailed t-test; $p<0.05$). There was no change in the capillary contacts in either group around the glycolytic type IIX fibres. There was no change in the capillaries per mm^2 of any fibre type in either group during the intervention period.

Table 5.2. Cardiovascular and metabolic changes following 11 week intervention (mean ± SEM).

	Training Group		Control Group		P Value	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Time**	**Group X Time**
Full Subject Cohort	**(n = 18)**		**(n=23)**			
$\dot{V}O_{2peak}$ (ml.kg^{-1}.min^{-1})	15.5 ± 0.8	16.7 ± 0.9*	16.3 ± 1.0	14.6 ± 0.8	0.509	0.001
Peak Workrate (W)	67 ± 5	80 ± 5†	69 ± 5	67 ± 6	0.018	0.004
Lactate threshold (W)	31± 3	42 ± 2†	29 ± 2	30 ± 2	0.000	0.002
Lactate threshold / Peak Watt %	45 ± 3	51 ± 2	46 ± 2	50 ± 3	0.012	0.393
Peak Lactates (mmol.L^{-1})	4.9 ± 0.3	5.7 ± 0.4	5.4 ± 0.6	4.7 ± 0.5	0.844	0.001
Quadriceps Strength (Nm)	106 ± 8	117 ± 9*	114 ± 7	115 ± 7	0.014	0.036
Hamstring Strength (Nm)	44 ± 3	59 ± 7*	48 ± 4	50 ± 5	0.029	0.090
Biopsy Subgroup	**(n = 7)**		**(n = 6)**			
$\dot{V}O_{2peak}$ (ml.kg^{-1}.min^{-1})	13.8 ± 1.1	15.5 ± 0.6*	14.8 ± 1.4	13.5 ± 1.3*	0.646	0.005
Peak Workrate (W)	61 ± 9	76 ± 10	60 ± 10	58 ± 11	0.057	0.021
Lactate threshold (W)	28 ± 4	45 ± 4†	25 ± 2	28 ± 4	0.003	0.012
Lactate threshold / Peak Watt %	44 ± 4	57 ± 3	49 ± 4	60 ± 3	0.027	0.467
Peak Lactates (mmol.L^{-1})	5.9 ± 0.8	6.8 ± 0.9$^{\#}$	4.7 ± 0.6	4.0 ± 0.6*	0.615	0.012
Peak Heart Rate (beats/min)	111 ± 5	114 ± 4	134 ± 8	122 ± 7	0.176	0.029
Quadriceps Strength (Nm)	92 ± 16	101 ± 17	110 ± 14	111 ± 11	0.069	0.201
Hamstrings Strength (Nm)	41 ± 6	51 ± 8	49 ± 9	52 ± 11	0.057	0.323

NB. In the full subject cohort the number of subjects for lactate data were 15 in the training group and 20 in the control group. In the biopsy subgroup lactate data was obtained from 6 in the training group and 5 in the control group n=5. *Denotes $p < 0.05$ vs. baseline; †Denotes $p < 0.01$ vs. baseline.

Table 5.3. Indices of muscle metabolism.

	Training Group (n = 7)		Control Group (n = 6)		P Value	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Time**	**Group X Time**
MAPR						
P+M	3.78 ± 0.76	4.91 ± 0.95*	4.79 ± 0.88	3.71 ± 0.63*	0.954	0.032
PC+M	1.83 ± 0.46	1.90 ± 0.28	2.28 ± 0.52	1.57 ± 0.37	0.304	0.215
α-KG	3.22 ± 0.92	3.79 ± 0.61	3.49 ± 0.64	2.85 ± 0.70	0.949	0.251
S+R	3.21 ± 0.94	4.00 ± 0.60	2.08 ± 0.59	2.75 ± 0.69	0.218	0.920
PPKM	5.02 ± 0.99	4.89 ± 0.73	4.26 ± 0.67	4.21 ± 0.82	0.855	0.933
Metabolic Enzymes						
CS	13.06 ± 1.03	18.24±1.63†	14.16 ± 0.75	15.25 ± 0.97	0.002	0.021
HAD	12.10 ± 1.00	17.16 ± 2.61	12.95 ± 1.75	12.55 ± 1.57	0.097	0.058
PFK	29.37 ± 1.45	29.19 ± 1.41	25.67 ± 2.24	28.80 ± 0.86	0.292	0.242
LDH	67.1 ± 16.7	65.0 ± 14.0	50.5 ± 6.7	36.1 ± 9.2	0.124	0.241

*Denotes $p < 0.05$ from baseline. †Denotes $p < 0.01$ from baseline.

Table 5.4. DEXA results prior to and following the 11 week intervention

	Training Group (n = 5)		Control Group (n = 6)		P Value	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Time**	**Group X Time**
Lean Mass (kg)	49.77 ± 4.27	49.69 ± 2.98	49.62± 2.12	50.04 ± 2.49	0.827	0.749
Lean Thigh Mass (g)	5.37 ± 0.48	5.51 ± 0.38	5.05 ± 0.44	5.12 ± 0.40	0.251	0.731

Table 5.5. Muscle fibre alterations following resistance-training intervention.

	Training Group (n = 6)		Control Group (n = 5)		P Value	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Time**	**Group X Time**
Fibre proportions (%)						
Type I	46.5 ± 3.0	47.5 ± 1.9	50.4 ± 3.4	44.7 ± 3.4	0.311	0.159
Type IIA	19.1 ± 2.0	17.9 ± 1.5	18.5 ± 1.6	21.4 ± 3.3	0.542	0.166
Type IIX	34.4 ± 2.1	34.6 ± 1.2	31.1 ± 2.6	33.9 ± 2.6	0.513	0.561
Muscle fibre areas (μm^2)						
Type I	2854 ± 272	3320 ± 227	3286 ± 156	3516 ± 342	0.081	0.520
Type IIA	3142 ± 417	2916 ± 265	2929 ± 143	3105 ± 352	0.904	0.344
Type IIX	2475 ± 281	2896 ± 250	2246 ± 261	2733 ± 197	0.030	0.857

Mean number of fibres used to compute fibre type proportions = 202 ± 15.

Table 5.6. Capillarity changes following 11-week intervention.

	Training Group (n = 7)		Control Group (n = 6)		P Value	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Time**	**Group X Time**
Capillary to fibre ratio	1.01 ± 0.05	1.17 ± 0.03*	1.15 ± 0.03	1.14 ± 0.03	0.063	0.039
Capillaries Contacting Individual Fibres						
Type I	3.02 ± 0.09	3.43 ± 0.12†	3.15 ± 0.11	3.26 ± 0.09	0.007	0.076
Type IIA	2.67 ± 0.18	2.86 ± 0.16	2.91 ± 0.11	2.64 ± 0.08	0.727	0.066
Type IIX	2.19 ± 0.16	2.36 ± 0.22	2.26 ± 0.05	2.41 ± 0.02	0.366	0.961
Capillaries per mm^2 of fibre area						
Type I	1154 ± 110	1145 ± 58	982 ± 54	999 ± 140	0.956	0.844
Type IIA	954 ± 72	1088 ± 72	1077 ± 53	935 ± 143	0.944	0.054
Type IIX	1005 ± 84	912 ± 69	1174 ± 208	987 ± 63	0.08	0.52

†Denotes $p < 0.01$ from baseline. NB. For capillaries per mm^2 of fibre area, training group (n = 6), control group (n = 4). Mean number of fibres used to compute capillary density = 197 ± 13.

5.4. DISCUSSION

5.4.1. Major Findings.

The major findings of the current study were that several indices of muscle oxidative capacity increased in the RT group and decreased in the C group. While CS has previously been observed to increase following RT in female CHF patients (Pu et al. 2001), this is the first study to examine the effects of RT on MAPR, a more robust indicator of muscle oxidative capacity, in CHF patients. $\dot{V}O_{2\ peak}$ was also increased in the training group and decreased in the control group following the resistance training program in the complete cohort as well as in the subgroup that underwent muscle biopsies. The observed changes in muscle oxidative capacity were strongly correlated to changes in $\dot{V}O_{2\ peak}$ (Figure 5.1) which has not been observed previously. These results may be clinically significant, as $\dot{V}O_{2\ peak}$ has previously been stated to be a significant predictor of survival in CHF (Anker et al. 1997c; Walsh et al. 1997). Thus the increases in $\dot{V}O_{2\ peak}$ observed in this study are a positive adaptation to the resistance training protocol and infer benefits in regard to prognosis and quality of life to the CHF patients group.

The resistance training protocol used in this study resulted in improvements in muscle strength in the overall training group and strong trends towards significant increases in strength in the sub group of patients who underwent a skeletal muscle biopsy (Table 5.2). However there was no relationship between changes in muscle strength and any muscle parameter measured.

5.4.2. Exercise Tolerance.

In the current study $\dot{V}O_{2\ peak}$ increased significantly in the RT group and decreased in the control patients. These results are significant as no previous study investigating the effects of RT

alone in CHF has reported an increase in $\dot{V}O_{2\ peak}$. Improved submaximal exercise tolerance and muscle efficiency (Hare et al. 1999; Pu et al. 2001) following RT protocols of between 10 and 11 weeks have been reported however, suggesting improvement in the functional capacity of the subjects. While the increase in $\dot{V}O_{2\ peak}$ in the RT group was significant in terms of improving functional capacity in CHF, the decrease in $\dot{V}O_{2\ peak}$ in subjects randomized to the control group has serious implications for CHF patients not involved in exercise training. Significant decreases in $\dot{V}O_{2\ peak}$ have not been reported previously in control subjects in training studies of CHF patients. However Kiilavuori et al. (2000) did report non significant increases in peak oxygen consumption in CHF patients following an endurance training protocol that became significant when compared to changes within the control group. This suggests mean decreases in $\dot{V}O_{2\ peak}$ in the control group although the data was not presented. The implications of these findings are that exercise training in CHF patients not only improves exercise tolerance but prevents a continued decline and should therefore be a treatment utilised in all CHF patients.

A trend towards an increase in muscle strength was noted in the training group but not the control group following the intervention period in the group of subjects who underwent a skeletal muscle biopsy. In the overall group of CHF patients involved in the study, significant improvements were noted in the strength of subjects in the resistance training but not the control group. These changes in strength are consistent with those of previous studies (Hare et al. 1999; Pu et al. 2001) utilizing resistance training in CHF patients. In addition to the increase in $\dot{V}O_{2\ peak}$ and muscle strength following the training protocol, significantly different responses to the intervention were found between the groups in the lactate threshold (Table 5.2). The results in the training group indicate an improved functional capacity following 11 weeks of RT (ie. the

work rate at which subjects can operate for an extended period of time), while the inactive controls exhibit some functional deterioration over the same period.

5.4.3. Muscle Oxidative Capacity.

In the present study a significant difference was noted in the group X time response to only one of the substrate combinations used for MAPR. This combination P+M, increased significantly in the training group and decreased in the control group over the intervention period. While this was the only substrate combination (of the five measured) to increase in response to the training stimulus, it was the one that was considered most likely to change prior to the study. This is because the P+M substrate combination simulates the effect of carbohydrate metabolism. During training patients exercised at heart rates approaching the peak heart rates they obtained during incremental exercise testing (>85% of peak) indicating that the exercise was of significant intensity. Given the intensity of the exercises and the rest periods between exercises it was expected that the majority of the increased demand for ATP resynthesis via oxidative mechanisms during the training sessions would be met by an up regulation of carbohydrate metabolism, as lipid metabolism has been demonstrated to be negligible at high exercise intensities (Romijn et al. 1993). Significantly, multiple regression analysis revealed changes in the substrate combination P+M to be significantly related to changes in $\dot{V}O_{2\ peak}$ (Figure 5.1; $p < 0.0001$) with the change in P+M accounting for 76% of the change in $\dot{V}O_{2\ peak}$ ($ml.kg^{-1}.min^{-1}$). Further evidence for the increase in skeletal muscle oxidative capacity is demonstrated by the increase in the activity of the enzyme CS in the training group following RT (Table 5.3). The other oxidative enzyme measured, HAD, is an enzyme involved in β-oxidation and hence can be used as a surrogate for the capacity of fat metabolism in the muscle. There was a trend towards a

group X time difference in the activity of this enzyme. Additionally, there was a significant increase in the activity of this enzyme in the training group. Interestingly this was not supported by any changes in the MAPR substrate combination of PC+M, which enables the measurement of the capacity of the skeletal muscle for fat oxidation more accurately.

There was a decrease in muscle oxidative capacity in the subjects randomized to the control group in this study. The reasons for this decrease are not immediately apparent. It is highly unlikely, although not impossible that the reduction in muscle oxidative capacity reported in the control patients was partially due to an age related decline in muscle oxidative capacity. While this phenomenon has been reported previously (Essén-Gustavsson and Borges 1986; Trounce et al. 1989; Houmard et al. 1998; Conley et al. 2000) other research has reported no differences in muscle oxidative capacity in similarly active young versus elderly subjects (Kent-Braun and Ng 2000; Rasmussen et al. 2003). Even in the event of muscle oxidative capacity decreasing with age it is unlikely that this would explain the reduction in the control subjects that was noted in the current study as the intervention period was only three months. While patients randomized to the control group were instructed to maintain their daily activities over the intervention period it is possible that there was a reduction in activity levels which may have resulted in reductions in muscle oxidative capacity. While activity levels in the weeks prior to the study and during the intervention were not quantitatively measured in the subjects randomized to the control group, these subjects were reminded regularly to maintain their normal levels of activity. Consequently this explanation appears unlikely to be the reason for the reductions in muscle oxidative capacity in the control group. A further explanation for the decrease may be continued progression of the disease. While low muscle oxidative capacity was not observed in this cohort of CHF patients as

described in chapter 4, the patients were non-cachectic with mild to moderate CHF at baseline. Previously cachexia has been related to the degree of exercise intolerance and reduced muscle function (Anker et al. 1997c). Progression of CHF eventually leads to cachexia, resulting in further reductions in exercise tolerance and muscle oxidative capacity. It is possible that during the intervention period the disease status of the control group continued to decline with the potential for the development of cachexia which would explain the reduction in muscle oxidative capacity. However the observations on muscle morphology and strength over this period do not support the hypothesis that the patients were becoming cachectic.

5.4.4. Skeletal Muscle Strength and Morphology.

Strength increased significantly in the quadriceps muscle group in the overall cohort in the RT but not C subjects ($p = 0.036$). There was also a tendency towards a similar increase in the hamstring muscle group ($p = 0.090$). While this finding has not been replicated in the biopsy patients there were mean increases in strength in both the quadriceps and hamstring muscle groups in the RT subjects. The failure to find significant differences in strength changes in the biopsy group is likely to be due to the small number of subjects investigated.

No between group changes were noted in either muscle fibre type proportions or muscle fibre areas in the subjects who underwent muscle biopsies (Table 5.5). While this result does not support the hypothesis that resistance training would result in increases in both strength and muscle fibre areas in CHF patients, it is entirely consistent with the small increases in strength observed in the RT group. The lack of increase in the cross-sectional area of muscle fibres and the lack of change in muscle fibre proportions following the training protocol suggest that the

small increases observed in muscle strength in the RT group were entirely due to enhanced neuromuscular facilitation. In this thesis I did not investigate the role of neuromuscular facilitation in increases in muscle strength in CHF. However it is widely acknowledged (Komi 1986; Hakkinen et al. 1988) that the initial increases in strength during resistance training in previously untrained individuals is due to improved neuromuscular facilitation. Significant adaptations in the muscle fibres are not observed in training periods of less than six weeks (Sale 1988). The current study utilised a training duration that has previously been demonstrated to be sufficient to observe muscle fibre adaptations in healthy young (Green et al. 1998) and elderly subjects (Frontera et al. 1990; Hepple et al. 1997). However these studies utilised training that included eccentric muscle contractions. As previously indicated resistance exercise incorporating an eccentric component is a more powerful stimulus for muscle hypertrophy than concentric exercise alone. The exercise training employed in this study involved predominantly concentric muscle contractions since it was thought that high eccentric loads may be deleterious to patients with CHF.

5.4.5. Resistance Training and Cardiovascular Parameters.

The resistance training protocol used in the current study utilised a hydraulic resistance training system and stair climbing combined with short duration (0.5 – 2 min) arm cycling and leg cycling. Long rest intervals were utilised between sets in the current study that enabled heart rates to return to within 10 $b.min^{-1}$ of the pre-exercise resting heart rate before the subject moved to the next exercise. As such the current protocol was distinct from either circuit or interval training, which utilise short rest intervals between exercises resulting in elevations in heart rate being maintained, and a mixture of aerobic, and strength training exercises. It is acknowledged

that although the training was classified as a resistance program, there may have been some overlapping aerobic effect due to the inclusion of the arm and leg cycling. The reason for the inclusion of these exercises was to provide additional strength exercises using cycling. Consequently subjects cycled for short durations and had prolonged rest periods before moving on to the next exercise.

Previously resistance exercise (weight training) in CHF patients has not been recommended due to a belief that it would further compromise left ventricular function. Some early studies of isometric exercise (as distinct from resistance exercise, which, in the context of this study is dynamic) demonstrated that LV function was compromised in functionally limited patients (NYHA Class III) who also had ischaemic heart disease (IHD) (Kivowitz et al. 1971). However, the deterioration was only transient. When aerobic training was supplemented with resistance exercise in men with coronary artery disease (CAD), there was a lower incidence of arrythmias and ischaemia (Daub et al. 1996) and LV wall motion abnormalities (Butler et al. 1987) during the resistance components of the programs than the aerobic components. Surprisingly, a combination of resistance and aerobic training in patients with CAD was superior to aerobic training alone in improving aerobic power and capacity, without increasing the incidence of cardiovascular complications (McCartney et al. 1991). This result was confirmed in the current study and has practical benefits for the CHF patient who wishes to follow an active lifestyle because it will enable the patient to exercise at the same relative percentage of $\dot{V}O_{2\,peak}$ with less fatigue and greater safety. The above studies indicate the importance of skeletal muscle strength to meet the challenges of whole body exercise and this should be emphasized for CHF patients.

5.4.6. Muscle Mass.

It was reported in chapter 4 (Figure 4.3) that muscle mass is a significant determinant of $\dot{V}O_{2\,peak}$ in CHF and may account for a portion of the exercise intolerance in this syndrome. However there were no training related changes in either lean thigh mass or muscle fibre cross-sectional area in the current study. Further, changes in lean thigh mass were unrelated to changes in $\dot{V}O_{2\,peak}$ (data not shown) following the training intervention suggesting that the majority of the improvements obtained in $\dot{V}O_{2\,peak}$ as a result of the training protocol were due to improvements in muscle oxidative capacity. It was also postulated that low muscle strength in CHF was related to the decreased type I fibre size (chapter 4) and therefore expected that any increase in type I fibre size that occurred over the training period would be related to increases in muscular strength. However, no relationship between changes in type I fibre size and muscular strength were found (data not shown). It is contended therefore that the increases observed in muscular strength in the training group in this study were primarily due to improvements in neuromuscular facilitation.

5.4.7. Muscle Fibre Proportions and Cross-Sectional Areas.

No changes in muscle fibre proportions were observed in the current study. This result is similar to that of Pu et al. (2001) who reported no alterations in muscle fibre proportions as a result of resistance training in a group of female CHF patients. This result was contrary to expectations as resistance training has previously been demonstrated to result in reductions in the proportions of type IIX muscle fibres combined with increases in the proportions of IIA fibres in healthy young (Green et al. 1998) and elderly (Hikida et al. 2000) males. The training programs employed in these studies however incorporated a significant eccentric component which may be important

for the stimulation of hypertrophy to occur (Hortobagyi et al. 2000). The failure to observe changes in fibre type proportions in the current study may be due to the purely concentric nature of the exercises performed. Pu et al. (2001) employed pneumatic resistance training equipment and hence their training stimulus consisted of predominantly concentric exercise.

There were no changes in the size of either type I or type IIA muscle fibres in either the training or control group in the current study (Table 5.5). However a time effect ($p = 0.03$) but no group X time effect was noted indicating a significant increase in the size of the type IIX muscle fibres in the overall subject cohort. While an increase in the size of type IIX muscle fibres in response to resistance training may be expected, there is no apparent reason to explain a similar increase in the control group. The increase in type IIX fibre size in the control group was caused mainly by large increases in the size of all fibre types of two control subjects. As histochemistry was performed on only five control subjects the low N may have been responsible for this anomalous observation.

5.4.8. Capillary Density.

Capillary to fibre ratio was increased in the training group following the intervention period which was accounted for by a significant increase in the number of capillaries contacting type I muscle fibres. Strong trends towards significance in the group X time response to capillary contacts to type I and type IIA fibres were also observed. Angiogenesis was stimulated around type I muscle fibres as a result of the training protocol however there was also a mean increase in type I fibre size in the RT group. Consequently, this did not equate to an increase in the number of capillaries per mm^2 of type I fibres in the RT group. Additionally there was a strong

tendency for a group X time effect in the number of capillaries per mm^2 of type IIA fibres (Table 5.6; $p = 0.054$) and a t-test revealed a trend towards an increase ($p = 0.07$) in capillaries per mm^2 in the RT group suggesting a possible increase in oxygen delivery to muscle fibres of this type. It should be noted however that the type IIA muscle fibres comprised approximately 20% of the total fibre population and hence any increase in capillarisation of these fibres will have limited effect on potential muscle blood flow. An this increase in capillary to fibre ratio (Frontera et al. 1990; Hepple et al. 1997) and capillary to fibre perimeter exchange index (Hepple et al. 1997) have both been reported following RT in healthy elderly male subjects. The increase in $\dot{V}O_{2\ peak}$ and skeletal muscle oxidative capacity that has been reported in this study without an associated increase in capillary density (capillaries/mm^2) provides further evidence in support of the previous contention (chapter 4) that capillary density is unlikely to be the limiting factor in exercise tolerance in CHF.

5.4.9. Metabolic Control

In Chapter 4 it was noted that muscle oxidative capacity was not a major determinant of $\dot{V}O_{2\ peak}$ in CHF, at least in the patients who participated in this study. Rather it appears that the capacity to activate aerobic metabolism is reduced in CHF patients due, potentially, to an earlier onset of anaerobic metabolism in incremental exercise in this group. As shown in Table 5.2 there was a significant increase ($p<0.01$) in the lactate threshold after training in both the entire cohort of RT patients and those who underwent muscle biopsy. It is possible the later onset of acidosis allowed greater activation of aerobic metabolism in this group after training and that this, in association with an increased muscle oxidative capacity, explains the increase in $\dot{V}O_{2\ peak}$ after training. This explanation for the increased $\dot{V}O_{2\ peak}$ in the RT group is not

supported entirely by the results obtained from C patients. There was a significant decline in $\dot{V}O_{2\ peak}$ ($p < 0.05$) and muscle oxidative capacity ($p < 0.05$) but no change in lactate threshold in either C group over the intervention period.

5.5. CONCLUSION

The major hypothesis of this study was that RT in CHF patients would result in improvements in $\dot{V}O_{2\ peak}$ and muscular strength and that these alterations would be due to alterations in the skeletal muscle morphology. In this chapter RT has been demonstrated to lead to increases in skeletal muscle oxidative capacity and to improvements in $\dot{V}O_{2\ peak}$ (Figure 5.1: P+M: $p < 0.0001$). The increased $\dot{V}O_{2\ peak}$ may be related to delayed onset of lactic acidosis as indicated by the increase in lactate threshold in the RT group. Muscular strength increased significantly in the quadriceps muscle group following resistance training and a tendency for a similar increase was also noted in the hamstring muscles. Muscle fibre type proportions were not changed following RT. Muscle fibre sizes in type I and type IIA muscle fibres were not altered by RT. Type IIX muscle fibre size increased in the combined (RT and C) biopsy group and as consequently this result can not be attributed to the training program. The improvements in strength in this study appear to be primarily due to improvements in neuromuscular facilitation as the RT program did not appear to stimulate the adaptations normally seen with RT in healthy individuals. Having now established the safety of this form of RT for CHF patients, it is postulated that a longer term, more intense RT program would not only be safe for these patients but would result in structural adaptations in skeletal muscle similar to those seen in healthy individuals.

CHAPTER 6. THE ROLE OF CHEMICAL MESSENGERS IN MUSCLE WASTING IN CHRONIC HEART FAILURE

6.1. INTRODUCTION

It is well established that skeletal muscle maladaptations accompany the remodeling of the myocardium that occurs with chronic heart failure (CHF) and that these alterations in skeletal muscle are closely associated with the exercise intolerance that strongly predicts prognosis in this condition (Sullivan et al. 1990; Duscha et al. 1999; Duscha et al. 2002). What are less well understood however, are the mechanistic factors responsible for the alterations in skeletal muscle structure and function in CHF.

A number of chemical messengers have been identified as mediators of muscle structure and function. For a discussion of some of these factors see chapter 2.9. Central among factors associated with muscle wasting is the cytokine TNF-α. Previously elevated systemic concentrations of cytokines such as TNF-α have been reported in CHF patients with cachexia (Levine et al. 1990; Anker et al. 1997b) leading to speculation about the effects of these cytokines on skeletal muscle. Elevated levels of TNF-α have also been associated with indicators of increased catabolism such as elevations in circulating cortisol (Anker et al. 1997a), elevated urea nitrogen (Levine et al. 1990), inhibition of IGF-1 expression (Fernandez-Celemin et al. 2002) and increases in iNOS expression (Kelly et al. 1996).

In chapter 4 it was reported that in the cohort of CHF patients examined in this thesis there were no differences in muscle oxidative capacity compared to similarly aged healthy control subjects. However there was a shift in muscle fibre proportions towards type IIX fibres which were the smallest fibres, and decreased type I muscle fibre size suggesting that the exercise intolerance in the CHF group was due in part to skeletal muscle atrophy. The resistance training undertaken in chapter 5 resulted in increases in $\dot{V}O_{2\ peak}$, muscular strength and muscle oxidative capacity in the CHF patients randomized to the training group suggesting a role for this type of exercise in CHF. While these differences and adaptations were noted in the previous experimental chapters the potential mechanisms leading to these changes were not described. Consequently this chapter examines certain chemical messengers involved in anabolic or catabolic pathways to determine whether they may have a significant role in the skeletal muscle changes observed in CHF or in the adaptations to resistance exercise training in CHF patients.

The aims of this study were twofold. The first was to determine the concentrations of a range of messenger molecules that have previously been implicated in an anabolic/catabolic response in skeletal muscle. These were compared in a group of CHF patients and healthy similarly aged control subjects with the intention of attempting to identify the mechanisms behind the muscle maladaptations that have previously been identified in CHF patients. The second aim of this study was to investigate any changes that occurred in the levels of these substances following 11 weeks of resistance training in a small group of CHF patients.

6.2. METHODS

A total of 20 CHF patients and eight healthy control subjects were investigated in this study. However data from only 15 of the CHF patients were used in the plasma analyses and 16 in the muscle analyses in this study. While the muscle analysis reflects the data of all but one of the patients who underwent a muscle biopsy (in the missing patient there was insufficient muscle available for NOS analysis), all CHF patients enrolled in the larger study had blood samples taken. Mechanical breakdown of a freezer in which plasma samples were stored resulted in the complete thawing of a number of samples and consequently these were not measured. Descriptive data for the separate components of this study are presented in Table 6.1. Similarly plasma samples from several of the healthy subjects also thawed preventing a complete data set for this group. Where correlations have been made between NOS and plasma variables only data from subjects who underwent both forms of testing have been used.

6.2.1. Blood Analyses

The collection and storage techniques used for the plasma were described in chapter 3. Frozen plasma samples were thawed and used for the measurement of cortisol, testosterone and TNF-α.

Table 6.1. Descriptive data for CHF patients and healthy control subjects.

	CHF Patients	Healthy	P
Plasma Study	N = 15	N = 5	
Age (yr)	67 ± 3	64 ± 5	0.643
Weight (kg)	79.7 ± 3.5	75.0 ± 4.5	0.440
BMI ($kg.m^{-2}$)	27.6 ± 1.3	26.6 ± 1.8	0.643
LVEF	27 ± 2	N/A	
NYHA class	2.1 ± 0.1	N/A	
$\dot{V}O_{2\,peak}$ **($ml.kg^{-1}.min^{-1}$)**	14.5 ± 0.8	28.2 ± 3.7	0.023
Peak Power (W)	59 ± 6	138 ± 21	0.016
Strength (Nm)	106 ± 10	124 ± 10	0.219
Muscle Study	N = 16	N = 8	
Age	67 ± 2	63 ± 4	0.337
Weight	84.3 ± 4.0	78.3 ± 3.3	0.257
BMI	28.4 ± 1.3	26.0 ± 1.2	0.192
LVEF	28 ± 2	N/A	
NYHA class	2.3 ± 0.1	N/A	
$\dot{V}O_{2\,peak}$ **($ml.kg^{-1}.min^{-1}$)**	15.1 ± 1.0	28.1 ± 2.3	0.0004
Peak Power (W)	66 ± 7	138 ± 13	0.0005
Strength (Nm)	110 ± 10	133 ± 6	0.068

6.2.1.1. Testosterone

Testosterone concentrations were determined in plasma samples using an I-125 labelled radioimmunoassay kit (DSL-4000, Diagnostic Systems Laboratory, Texas). Briefly standards and samples were added to precoated tubes in duplicate containing an antibody for testosterone. Following this step a solution containing testosterone bound to radiolabelled iodine (I-125) was added to the tubes where it was bound to antibodies that remained unbound following the

addition of the standards and samples. Following an hour of incubation at 37°C, the tubes were decanted and counted in a gamma counter (Packard Bell, Cobra II Auto-Gamma, Packard Instrument Company, USA). A log fit curve was derived from the standard results using inbuilt software (Auto-Gamma Counting Series Software) and the concentration of testosterone in the unknown samples was determined from the standard curve.

6.2.1.2. Cortisol

The concentration of cortisol in plasma samples was determined using a radioimmunoassay kit (DSL-2000, Diagnostic Systems Laboratories, Texas). Briefly, standards and samples were added in duplicate to tubes that had been precoated with an antibody for cortisol. Following this step a solution containing cortisol bound to radiolabelled iodine (I-125) was added to the tubes where it was bound to antibodies that remained unbound following the addition of the standards and samples. Following a 45-minute incubation at 37°C, the tubes were decanted and counted in a gamma counter (Packard Bell, Cobra II Auto-Gamma, Packard Instrument Company, USA). A log fit curve was derived from the standard results using inbuilt software (Auto-Gamma Counting Series Software) and the concentration of cortisol in the unknown samples was determined from the standard curve.

6.2.1.3. TNF-α

Tumor Necrosis Factor - α (TNF-α) was measured in plasma samples of 15 CHF patients and five healthy control subjects. A chemiluminescent immunoassay kit (QTA00, Quantiglo® Human TNF-α Chemiluminescent immunoassay, R&D systems, Texas) was used to determine the concentration of TNF-α in each sample. The assay employed a quantitative sandwich

enzyme immunoassay which was run at 21°C on a luminometric microplate reader (Ascent Fluoroskan FL; Labsystems). Briefly, microplate wells were precoated with a monoclonal antibody specific for TNF-α. Samples and standards were pipetted into the wells where TNF-α was bound by the immobilized antibody. Washing agents were used to remove any unbound material from the wells and an enzyme linked polyclonal antibody specific for TNF-α was then added to the wells. Following a wash to remove any unbound antibody-enzyme reagent a luminol peroxide solution was added to the wells which caused light to be produced in proportion to the amount of TNF-α bound in the initial step. TNF-α concentrations were quantified on a standard curve derived from the light produced by standards in the range of 0 to 7000 pg/mL.

6.2.2. Nitric Oxide Synthase (NOS)

Inducible NOS (iNOS) and neuronal NOS (nNOS) were measured in muscle taken from the vastus lateralis at rest and stored in liquid nitrogen until assayed. For details of the biopsy procedure see chapter 3.5.1. Details of the assay procedure are reported below.

6.2.2.1. Extraction of Total Protein.

Tissue was weighed and diluted 1:20 (w:v) in an extraction buffer containing 10ml of 50mM Tris HCl (pH 8.0), 50mM NaCl and 5mM $CaCl_2$, combined with 1μM $ZnSO_4$, 1.5mM NaN_3 and 0.5% phenylmethylsulfonyl (PMSF). Samples were homogenised for 3 × 20 seconds on ice then 0.1% Triton X-100 was added. Samples were agitated at 4°C for 4 hours then centrifuged at 12000g for 5 minutes at 4°C. Supernatant was removed for protein determination.

6.2.2.2. Determination of Protein Concentration.

Protein concentrations were determined using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA).

6.2.2.3. Western Blot Analysis.

Steady state protein levels were determined using western blot analysis using Bio-Rad instructions. Briefly, samples were run along side pre-stained markers (Bio-Rad laboratories, Hercules, CA, USA) in SDS-polyacrylamide (SDS-PAGE) gels containing 30% acrylamide (7% separating gel and 4% stacking gel), 10% SDS, 10% Ammonium Persulfate (APS), water and tetra-methyl-ethylenediamine (TEMED and analysed by electrophoresis for 90 minutes at 30 milliamps in a running buffer containing 25mM Tris Base, 192mM Glycine and 0.1% SDS (pH 8.0). Gels were then washed for 15 minutes in transfer buffer (25mM Tris Base, 192mM Glycine and 10% methanol) at room temperature while agitating.

Proteins were transferred onto 0.45μm nitrocellulose (Bio-Rad Laboratories, Hercules, CA, USA) at 0.25amps for 3½ hours at room temperature. Membranes were blocked with 3% skim milk in solution with 0.5M Tris/HCl (pH 7.4) and 0.15M NaCl for 1hour at room temperature while agitating and then washed for 2×10 minutes in buffer A (0.15M NaCl, 0.01M NaH_2PO_4, 0.01M Na_2HPO_4 and 0.05% tween) and 1×5 minutes in buffer B (0.15M NaCl, 0.01M NaH_2PO_4, 0.01M Na_2HPO_4) followed by incubation in primary antibody overnight (Anti - iNOS mAb and Anti - nNOS mAb; Transduction Laboratories; Lexington, KY, USA). Following incubation the wash steps were repeated and followed by 2½ hour incubation with a secondary antibody (Goat-anti mouse; Biorad; Hercules, CA, USA) and positive control (Transduction Laboratories; Lexington, KY, USA). After a final series of washes the signals were detected

with enhanced chemiluminescence (ECL) (Amersham Pharmacia Biotech, Buchinghamshire, England, UK).

6.2.2.4. Protein Quantification from Western Blots.

Western blot bands were quantified using Optimus 6.51 analysis software (Media Cybernetics, Silver Spring, MD, USA). Software was densitometrically calibrated to the Linotype-Hell scanner (Saphire Ultra 2, Linotype-Hell, Heidelberg, Germany) using an AGFA transmissive step wedge. After tracing the band area a pre-written macro normalised for the background density and derived the final result as the product of density and area.

6.2.3. Statistics

All statistics were performed using either the Microsoft Excel or SPSS software packages. Results obtained from the CHF patients and healthy control subjects at baseline were compared using student's unpaired T-tests. To compare the effects of training or inactivity, results were analysed using a 2 way ANOVA.

6.3. RESULTS

No significant differences were found between CHF patients and healthy control subjects in either the plasma variables (Table 6.2) or in iNOS or nNOS expression (Table 6.3). A trend towards elevated plasma concentrations of TNF-α were observed in the CHF compared to the healthy subject group.

Table 6.2. Plasma cortisol, testosterone and TNF-α in healthy subjects and CHF patients.

	CHF (n = 15)	Healthy (n = 5)	p
Cortisol	11.17 ± 1.40	11.86 ± 3.44	0.86
Testosterone	4.74 ± 0.36	3.42 ± 0.79	0.18
Cort:Test Ratio Males only	2.40 ± 0.34	3.03 ± 0.98	0.57
TNF - α	1.99 ± 0.33	1.01 ± 0.41	0.09

Data is presented as mean ± SEM.

Table 6.3. Skeletal muscle iNOS and nNOS expression.

	CHF (n = 15)	Healthy (n = 5)	p
INOS	24.74 ± 3.11	26.82 ± 4.40	0.707
NNOS	12.70 ± 0.94	14.37 ± 1.89	0.436

Data is presented as mean ± SEM.

The relationship between TNF-α and $\dot{V}O_{2\,peak}$ was low in the cohort of CHF patients (r = 0.347; p = 0.17) used in this study. No significant relationships were noted between plasma TNF-α levels and indices of either muscle oxidative function or muscle morphology. Complete tables of correlations between chemical messengers measured in this chapter and exercise and muscle variables are presented in Appendix III.

A trend towards an inverse correlation was observed between the cortisol:testosterone ratio and the MAPR substrate combination P+M (r = 0.569; p = 0.068). There was a trend towards an inverse correlation between skeletal muscle iNOS expression and muscle strength in the CHF patients at baseline (p = 0.127). A trend towards a positive correlation was also observed between nNOS expression and skeletal muscle type I fibre size in the CHF patients (p = 0.106).

A significant correlation was observed between the cortisol:testosterone (C:T) ratio and muscle nNOS protein expression ($r = 0.616$; $p<0.05$).

There was a time effect but no group X time effect in plasma testosterone (Table 6.4). No changes in plasma concentrations of cortisol or TNF-α were noted. Skeletal muscle iNOS and nNOS were unaltered following the training stimulus (Table 6.5).

Table 6.4. Alterations in plasma cytokine and neurohormonal factors following 11 week intervention.

	Training Group (n = 7)		Control Group (n = 6)		P Value	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Time**	**Group x Time**
Cortisol	12.40 ± 1.65	12.20 ± 1.97	11.52 ± 2.68	11.13 ± 1.86	0.796	0.932
Testosterone	4.73 ± 0.55	5.09 ± 0.50	4.47 ± 0.65	4.60 ± 0.66	0.011	0.199
C:T Ratio	2.7 ± 0.3	2.4 ± 0.4	2.9 ± 0.6	2.5 ± 0.3	0.212	0.913
TNF - α	1.52 ± 0.39	1.62 ± 0.43	2.54 ± 0.82	2.36 ± 0.89	0.854	0.414

Data is presented as mean ± SEM.

Table 6.5. Changes in muscle iNOS and nNOS over the 11 week intervention

	Training Group (n = 6)		Control Group (n = 5)		P Value	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Time**	**Group x Time**
NNOS	14.74 ± 2.94	13.10 ± 2.79	15.59 ± 4.64	13.04 ± 5.03	0.479	0.876
INOS	24.33 ± 6.07	22.38 ± 4.64	30.75 ± 5.89	25.78 ± 4.88	0.204	0.564

Data is presented as mean ± SEM.

6.4. DISCUSSION

6.4.1. Major Findings

In this study there was a strong tendency towards a difference in TNF-α between CHF patients and healthy control subjects. This indicates that it may possibly be a causative factor in the significant differences between the groups in exercise tolerance, muscle strength and other muscle parameters. However no significant difference was observed between the CHF patients and healthy control subjects in the concentration or expression of any of the messenger molecules measured.

There were no significant changes in the concentration of any of the chemical messengers measured following 11 weeks of resistance training in a subgroup of CHF patients. As previously indicated however (chapter 5) there were significant improvements in $\dot{V}O_{2\ peak}$, muscle oxidative capacity and capillary to fibre ratio following the training intervention. Previous studies (Riede et al. 1998; Alloatti et al. 2000; Sharma et al. 2000; Cicoira et al. 2001a) have associated the reduced exercise tolerance in CHF patients to alterations in the expression or secretion of many of the molecules that were measured in the present study. Consequently it was expected that differences in some or all of these molecules would be observed between the CHF patients and the healthy control subjects. Additionally it was hypothesised that if the resistance training protocol resulted in alterations in exercise tolerance and skeletal muscle metabolic and/or morphologic properties these would be correlated to changes in many of the compounds measured in this study.

A series of regressions were performed in order to attempt to determine the relationships between the signalling molecules measured in this study and indices of exercise tolerance, muscle metabolism and muscle morphology. Despite the lack of difference between the CHF patients and healthy subjects there were significant correlations between testosterone and $\dot{V}O_{2\ peak}$, ($r = 0.583$; $p<0.05$) and testosterone and capillary to fibre ratio ($r = 0.665$; $p<0.05$) in the CHF patients but not the control subjects. The only other significant correlation occurred between nNOS expression and LDH activity. These correlations will be discussed in detail subsequently.

6.4.2. Differences in Subject Cohort Between this Study and Previous Studies.

CHF patients in the current study displayed similar levels of exercise impairment as have been reported by others (Sullivan et al. 1990; Clark et al. 1996) however they did not exhibit a number of the skeletal muscle maladaptations that have been previously reported in CHF patients (Sullivan et al. 1990; Massie et al. 1996; Duscha et al. 1999). The reasons for this are not entirely clear but may have to do with differences between the subject cohort used in the current study and that used in similar studies. As discussed in chapter 4 the CHF patients included in this thesis are representative of the patients with systolic heart failure who attend a heart failure clinic, not having been selected because of cachexia, severe functional impairment or referred specifically for cardiac transplantation. Some of the differences between the cohort of CHF patients used in this study and others are discussed below.

The CHF patients examined in the current study had a mean age (Table 6.1) older than that of the patients examined in many similar studies (Anker et al. 1997a; MacGowan et al. 1997; Zhao

and Zeng 1997; Riede et al. 1998). Aging is associated with increases in circulating levels of inflammatory markers (Bruunsgaard and Pedersen 2003) and decreased anabolic/catabolic balance (Izquierdo et al. 2001). Consequently, differences in the mean age of groups in previous studies and between CHF patients in previous studies and the current study could possibly influence some of the differences noted in the results obtained between the current study and previous work.

CHF patients recruited for the current study were all in NYHA functional class II and III but there were many more patients in NYHA class II than III (Table 6.1). Studies that have investigated the relationship between CHF and different chemical messengers previously, have either used patients from a wider range of functional classes and exercise tolerance (Anker et al. 1997a; Anker et al. 1997b; Anker et al. 1999b; Cicoira et al. 2001a), patients who are cachectic (Anker et al. 1997a; Anker et al. 1997b) or patients with more severe CHF (Levine et al. 1990; Zhao and Zeng 1997).

CHF patients recruited for the current study were not specifically identified regarding their cachectic status however only 2 of the subjects exhibited a BMI of < 24 kg/m^2 which along with a non intentional weight loss of >5 kg over a period of at least six months is one of the commonly used indicators of cachexia (Anker et al. 1997a). Consequently it is unlikely that any of the CHF patients in the current study were cachectic.

The CHF patient details noted in this section highlight the problems associated with comparing the results of the current study with those of previous researchers. These differences mean that

the results obtained in this study may be attributable to the population of CHF patients used in this study only and are not necessarily representative of the wider CHF population.

6.4.3. Tumor Necrosis Factor α

A strong trend towards a difference in TNF-α ($p = 0.09$) was noted between the CHF patients and healthy control subjects in the current study. Given that this trend was noted in such a small sample population (15 CHF; 5 healthy) it is highly likely that a full data set (45 CHF; 8 healthy) would have yielded significant differences. Consequently the results for TNF-α will be discussed as if they were significantly different. Despite differences in TNF-α between CHF patients and healthy control subjects, and the previously reported relationship between circulating TNF-α and the degree of exercise intolerance in CHF patients (Cicoira et al. 2001a), only a weak inverse relationship was noted between exercise tolerance and TNF-α in the present study ($p = 0.17$). This may be due to the clinical status of patients in the current study. Patients with the greatest elevations in TNF-α when compared to healthy subjects tend to be those who have the most severe heart failure or who are classified as cachectic. As already discussed, patients in the current study had only mild to moderate CHF and did not appear to be cachectic.

There is ample evidence in the literature that TNF-α influences skeletal muscle via either direct or indirect mechanisms. Previously, elevated systemic concentrations of cytokines such as TNF-α have been reported in CHF patients with cachexia (Levine et al. 1990; Anker et al. 1997b) leading to speculation about the effects of these cytokines on skeletal muscle. Elevated levels of TNF-α have also been associated with indicators of increased catabolism such as elevations in circulating cortisol (Anker et al. 1997a), inhibition of IGF-1 expression (Fernandez-Celemin et

al. 2002) and increases in iNOS expression (Kelly et al. 1996). While these effects are likely to have an indirect effect on skeletal muscle wasting, elevated levels of TNF-α may also have a more direct catabolic effect through the initiation of a cascade resulting in the upregulation of the ubiquitin proteasome pathway. This pathway was discussed in detail in chapter 2.9.2. It appears certain that elevated circulating levels of TNF-α will have a role in both the direct and indirect initiation of muscle protein catabolism, and therefore muscle wasting leading to cachexia, however no relationship was seen between TNF-α concentration and any of the muscle variables measured in the current study. As previously discussed (chapter 4), muscle wasting may contribute to but is not the major cause of exercise intolerance in CHF patients. Consequently, while it is acknowledged that TNF-α is likely to be a causative factor in exercise intolerance due to muscle wasting, other factors are clearly also involved.

Contrary to the results of previous studies that have reported associations between TNF-α and iNOS (Kelly et al. 1996; Alloatti et al. 2000) or catabolic/anabolic ratios (Anker et al. 1997b) no such correlations were noted in the present study. Either the clinical status of the current cohort of CHF patients or the sample number may be reasons for the lack of association between TNF-α and other mechanistic factors. However it is unlikely given the degree of lack of association between these factors, that significant correlations would have been observed in this study regardless of the number of subjects.

6.4.4. Cortisol and Testosterone

There were no differences between plasma cortisol or testosterone concentrations between the CHF patients and healthy control subjects at baseline. Neither was there a difference in the C:T

ratio, an indicator of relative catabolic balance. In the CHF patient group no relationship was noted between the C:T ratio and either $\dot{V}O_{2\,peak}$ or quadriceps muscle strength. Interestingly there was a trend towards a significant inverse correlation between the C:T ratio and the MAPR substrate combination P+M ($p = 0.068$). While the reason for such a relationship is not immediately obvious it is possible that the increased C:T ratio induced the atrophy of the type I oxidative muscle fibres that was reported in chapter 4. If this was the case however, a significant correlation between C:T ratio and type I muscle fibre size might be expected but this did not occur. Further, it would be expected that MAPR would be reduced in the CHF patients. As reported in chapter 4, this was not the case in the cohort of CHF patients used in this thesis and consequently it is difficult to explain a relationship between C:T ratio and MAPR.

6.4.5. Skeletal Muscle iNOS Expression

An increased expression of muscle iNOS has been reported in the skeletal muscle of patients with severe heart failure (Riede et al. 1998) and would be expected to result in excessive production of NO with a range of deleterious effects on skeletal muscle as described in chapter 2. This was not found to be the case in the CHF patients examined in this study. Riede et al. (1998) used skeletal muscle taken during vastus lateralis muscle biopsies in CHF patients in NYHA functional class III. The authors speculated that the increased iNOS expression that they observed was due to elevated systemic and/or local cytokine activation in the CHF patients although they did not measure this. If elevated iNOS expression in CHF is the result of elevated cytokine concentrations as has been suggested by Riede et al. (1998) and others (Schulze et al. 2002), it explains the lack of significant differences in iNOS expression between the CHF patients and healthy control subjects in the current study.

A potential limitation in the study of Riede et al. (1998) was that the healthy controls were an average of eight years or 15% younger than the CHF patients they were compared with. The expression of nNOS increases with aging in skeletal muscle cells (Capanni et al. 1998; Richmonds et al. 1999). Likewise iNOS expression has been demonstrated to increase in brain tissue with aging (Hilbig et al. 2002; Law et al. 2002). If iNOS were similarly affected in muscle cells this may have confounded the results observed by Riede et al. (1998). The healthy subjects in the current study had a mean age that was four years or 6% lower than the CHF patients. This would have been expected to exacerbate any difference in iNOS expression between the groups, further delineating the lack of difference that was observed.

Although there was no significant difference between CHF patients and healthy control subjects in iNOS expression in the current study, a significant inverse correlation was observed between iNOS expression and quadriceps muscle strength in the CHF patients ($p = 0.05$). Increased iNOS expression may be expected to result in elevations in the tissue concentration of NO, which has a number of functions within skeletal muscle. As previously indicated (section 2.9.4.1), at high concentrations NO has been demonstrated to attenuate the contractile force of skeletal muscle (Murrant et al. 1997) which is consistent with the finding of an inverse relationship between iNOS expression and muscle strength in CHF patients in the current study. Nevertheless it must be acknowledged that the effects of NO on contractile function are small and may not be of major physiological significance (Kaminski and Andrade 2001). Additionally muscle strength increased following 11 weeks of resistance training (reported in chapter 5) but there was no change in iNOS expression following the training protocol. While other mechanisms may have a

role in the adaptations to training in CHF, the absence of a change in iNOS expression suggest that it is not a causative factor in skeletal muscle dysfunction in CHF.

Previously NO has been reported to inhibit mitochondrial respiration in rat hepatocytes (Stadler et al. 1991) and vascular smooth muscle cells (Geng et al. 1992). In the current study no relationship was noted between iNOS expression and either $\dot{V}O_{2\ peak}$ or any measure of skeletal muscle oxidative capacity. However the lack of difference in iNOS expression between the CHF patients and healthy control subjects in this study indicates normal iNOS expression in the patient group and therefore this lack of relationship was not unexpected.

6.4.6. Skeletal Muscle nNOS Expression

There was no difference in nNOS expression between the CHF patients and healthy control subjects in the current study. nNOS is expressed in skeletal muscle tissue and is thought to play a role in the healthy activity of skeletal muscle. No relationship was noted between nNOS expression and any index of exercise tolerance in the CHF patients. However a negative relationship was noted between nNOS protein expression and the activity of the LDH enzyme ($p<0.05$). A trend towards a significant relationship was also noted between nNOS expression and type I fibre size ($p = 0.106$). While these correlations are interesting it is not immediately apparent what causative mechanism might be responsible for this relationship.

6.4.7. Relationship between Mechanistic Factors

Elevated levels of TNF-α and other cytokines are associated with increases in iNOS expression in CHF patients (Schulze et al. 2002). No such relationship was found in the current study.

However in the current study no significant difference was noted in TNF-α between CHF patients and healthy control subjects. This study recruited patients who had mild to moderate CHF and while a number of differences were noted between the groups in terms of muscle morphology, no differences were noted in either muscle oxidative capacity or mechanistic variables. Previously TNF-α has been correlated with exercise intolerance (Cicoira et al. 2001a), NYHA functional class (Torre-Amione et al. 1996) and cachexia (Levine et al. 1990; Anker et al. 1997a) in CHF patients. While no such relationships were observed in the current study this may be due to a range of factors including but not limited to clinical status. Additionally, although the CHF patients in the current study exhibited significant exercise intolerance, they did not exhibit all of the skeletal muscle maladaptations that have been reported previously in CHF (discussed in chapter 4).

6.4.8. Limitations

Some limitations of the current study include the small number of subjects that were used to determine both muscle and plasma variables. As previously mentioned the number of subjects measured for plasma variables (TNF-α, cortisol, testosterone) was severely reduced due to the failure of a freezer in which many of the samples were stored. Although no such problem was encountered with the muscle samples used to determine NOS expression, only a subgroup of patients in the larger study cohort elected to undergo muscle biopsy testing. nNOS and iNOS were the final variables measured with the muscle taken from these patients and in one patient the muscle had been exhausted prior to NOS testing. Consequently there were fewer samples available for these assays than for other muscle testing. A final limitation of the current study was the use of patients with only mild to moderate CHF. Previous studies investigating

mechanistic factors involved in the muscular maladaptations in CHF have recruited CHF patients either from a wide range of prognoses or have compared severe or cachectic CHF patients with healthy control subjects.

6.5. CONCLUSION

The major finding of the current study was that there was a tendency towards differences between plasma TNF-α concentrations in CHF patients and healthy control subjects which may be a cause of the exercise intolerance reported in CHF. However no differences were observed between the groups in skeletal muscle iNOS or nNOS expression or plasma concentrations of cortisol or testosterone either. These results contrast with those of studies that have investigated these mechanistic factors in CHF patients previously. Eleven weeks of RT was found to increase plasma testosterone concentrations without altering catabolic/anabolic balance. Likewise no other mechanistic factor was altered as a result of the RT intervention. It is possible that the lack of consistency between the results of the current and previous studies were due to limited sample size or patient prognosis. TNF-α appears to negatively influence exercise tolerance in CHF through both direct and indirect effects on muscle wasting eventually leading to cachexia. However factors other than muscle wasting are clearly also involved in the exercise intolerance and require further investigation.

Two separate pathways leading to reductions in exercise tolerance are likely to exist in CHF. The initial decreases in $\dot{V}O_{2\,peak}$ occur early in heart failure and may not involve muscle wasting. It is unlikely that increases in circulating TNF-α concentration will have a role in this pathway. The second pathway, which has been investigated in this thesis, involves additional

decrements in $\dot{V}O_{2\,peak}$ due to alterations in muscle fibre type proportions and size. It appears likely that TNF-α has a major role in this pathway.

CHAPTER 7. THE ROLE OF THE ACE GENE IN REDUCED EXERCISE TOLERANCE IN CHF

7.1. INTRODUCTION

The angiotensin converting enzyme (ACE) catalyzes the production of angiotensin II and renders bradykinin inactive. A polymorphism in intron 16 of the ACE gene is characterized by the presence (insertion - I) or absence (deletion - D) of the 287-bp Alu sequence. It has been estimated that between 20 and 50% of the variation in serum ACE concentration may be due to this polymorphism, with the highest levels in individuals carrying the DD genotype (Rigat et al. 1992).

Previously ACE DD genotype has been related to an increased incidence of myocardial infarction (Cambien et al. 1992) and early onset coronary heart disease (Ruiz et al. 1994) independent of classical risk factors. Increased risk of secondary events following myocardial infarction have also been linked to the D allele (Yoshida et al. 1999). However, the relationship between the ACE D allele and left ventricular hypertrophy more generally is less certain (Lindpaintner et al. 1996).

Coronary artery disease, myocardial infarction and left ventricular hypertrophy are all associated with chronic heart failure (CHF). The major symptoms of CHF are fatigue on exertion and breathlessness (Clark et al. 1996) and poor exercise capacity is a strong and independent prognosticator in CHF (Bittner et al. 1993). It has been demonstrated that aerobic exercise training improves $\dot{V}O_{2\,peak}$ and exercise tolerance (Coats et al. 1992) in these patients.

Several studies have suggested that ACE genotype may have a role in adaptations to exercise as individuals with the I allele have been reported to have a greater suitability for endurance exercise while individuals with the D allele may be better suited to resistance training (Montgomery et al. 1997; Gayagay et al. 1998; Abraham et al. 2002). Skeletal muscle parameters such as fibre type proportions and metabolic enzyme activities have also been linked to suitability for different types of exercise (Costill et al. 1976; Tesch et al. 1984; Tesch et al. 1987; Coyle et al. 1991; Esbjornsson et al. 1993). As previously discussed, skeletal muscle is a highly plastic tissue that adapts readily to training stimuli (Klausen et al. 1981; Staron et al. 1991; Dawson et al. 1998) resulting in improved exercise performance. Conversely, skeletal muscle maladaptations contribute to the reduced exercise tolerance observed in CHF patients. Recently Zhang et al. (2003) reported subjects with the II allele to have higher percentages of type I muscle fibres and lower percentages of type IIX fibres than subjects homozygous for the D allele. Consequently ACE gene polymorphism is worth investigating in CHF patients. Further, if ACE genotype is a predisposing factor in the response to exercise of various forms, it may be a worthwhile measurement, allowing CHF patients to be prescribed forms of exercise that they are most suited to.

Given the possible mechanisms by which ACE gene I/D polymorphism may influence the severity of CHF and may also have a role in fitness and skeletal muscle parameters, the aim was to investigate possible associations between ACE genotype and exercise tolerance and skeletal muscle parameters in patients with CHF. Further a comparison of the response to 11 weeks of

resistance training was conducted in a small group of CHF patients to see if ACE genotype had an effect on trainability.

7.2. METHODS

7.2.1. Subjects. 32 men and six women with stable CHF participated in this study. Descriptive characteristics of the subjects are presented in Table 7.1.

Table 7.1. Descriptive characteristics of CHF patients. (Data are mean ± SD)

	Male	Female	Total Group
N	32	6	38
Age (yrs)	65 ± 9	57 ± 20	64 ± 12
Height (m)	1.73 ± 0.07	1.62 ± 0.08	1.71 ± 0.08
Weight (kg)	81.1 ± 14.6	92.4 ± 23.2	82.9 ± 16.4
BMI (kg/m)	27.2 ± 4.8	35.1 ± 7.1	28.5 ± 5.9
NYHA Class	2.3 ± 0.4	2.3 ± 0.5	2.3 ± 0.5
LVEF	27 ± 7	34 ± 4	28 ± 7

7.2.2. Exercise Testing

The exercise testing involved a cycle ergometer test using an incremental protocol. Quadriceps muscle strength testing was performed using an isokinetic dynamometer with a movement arm speed set at 60° second. These tests were conducted at the Austin and Repatriation Medical Centre using methods that have been described previously (Chapter 3).

7.2.3. Resistance Training

A subgroup of 19 CHF patients (16 male:3 female) underwent an 11-week low intensity resistance-training program following baseline testing. The details of the resistance training protocol have been described in detail in Chapter 3. Characteristics of the training and control groups of patients are presented in Table 3. One subject did not return for endpoint testing.

7.2.4. Blood Sampling

Blood samples for ACE genotype analysis were collected from the antecubital vein using a direct needle puncture technique. The blood was collected in heparin coated tubes and stored on ice for transport to the laboratory where the DNA extraction technique was performed.

7.2.5. ACE Genotyping

7.2.5.1. DNA Extraction.

Fresh blood samples were spun at 4000 rpm at 4°C. White blood cells were harvested from the buffy coat, stored in freezer tubes and frozen until ready to assay. DNA was extracted from white blood cells using standard salting-out methods (Miller et al. 1988). These methods involved diluting white blood cells in a solution containing 3M NaCl and 0.3M trisodium citrate at pH 7.0. The resulting solution was then shaken to completely lyse cells and centrifuged at 4000 rpm for 5 minutes. The pellet was harvested and the step repeated until all evidence of haemoglobin was removed. The final pellet was then resuspended in 2.5mL of 2M sodium actetate (pH 7.0) and shaken vigorously. 250μL of 10% sodium dodecyl sulphate (SDS) was

added and the solution was inverted until it became viscous. 2.75mL of Phenol Chloroform Isoamyl Alcohol was added and the resultant solution was mixed thoroughly to generate and maintain an emulsion. The emulsion was then centrifuged and the clear and clean aqueous phase was collected into a new labelled tube. The organic phase was re extracted with 1.25mL of 2M sodium acetate re centrifuged to improve the yield. DNA was precipitated from the aqueous phase by the addition of 100% ethanol. The solution was centrifuged again and the supernatant discarded. This process was repeated and the final pellet allowed to dry overnight at room temperature. The following morning the pellet was resuspended in sterile distilled water and the pelletised DNA permitted to dissolve.

7.2.5.2. PCR Amplification.

Each genotype was identified by polymerase chain reaction (PCR) amplification of a sequence from intron 16 of the ACE gene (Montgomery et al. 1997).

Samples were diluted 1:20 in a cocktail containing 10x PCR buffer II, 3mM $MgCl_2$, dNTP (#n808 0007; PEBiosystems), Primer mix, Amplitaq Gold (10x PCR buffer, $MgCl_2$, taq polymerase; #n808 0241; PEBiosystems).

The PCR was run on a program thermocycler using the following setup. The denaturation phase was run at 94°C for 1 minute. The annealing phase consisted of 30, 1minute cycles at 58°C and the extension phase consisted of a 2 minute cycle at 72°C followed by a 10 minute cycle at 72°C.

7.2.5.3. Gels.

Subsequently, alleles (490 bp: I and 190 bp: D) were differentiated on an agarose gel by ethidium bromide staining (Montgomery et al. 1997). The agarose gel was prepared by adding 3g agarose to 150mL 0.5M Tris-Borate-EDTA buffer (TBE) and mixing thoroughly. The mixture was microwaved for short durations and mixed while warm to ensure the agarose dissolved completely. Once the agarose was dissolved 7μL of ethidium bromide was added to the solution and mixed thoroughly. After the solution had been left to stand for a short period of time the gel was poured and left to set for an hour. Samples were prepared by adding 5μL of loading buffer containing 0.025g bromophenol blue and 4g sucrose in 10mL of H_2O to 20μL of a 1:10 dilution of DNA. Wells were loaded with 25μL of prepared samples. Loaded gels were floated in (0.5M TBE) and run at 100V.

7.2.6. Muscle Biopsy

A subgroup of patients (n = 14; 13 males, 1 female) underwent a skeletal muscle biopsy. The details of this technique have been described previously (Chapter 3).

7.2.7. Muscle Analyses

Muscle obtained from the biopsy underwent testing for MAPR, metabolic enzymes activity, muscle fibre type and capillary density. The techniques used to measure these variables have been described in chapter 3.

7.2.8. Statistical Analysis

A χ^2 test was used to determine whether the observed genotype frequency was in Hardy Weinberg equilibrium. One-way ANOVA was used to test associations between $VO_{2\ peak}$, peak power output, quadriceps muscle strength, a range of muscle parameters and ACE genotypes in CHF and the differences in the training response (except for muscle parameters) between genotypes. Where relevant Newman-Kuels post-hoc testing was used to assess differences between specific genotype groups. T-tests were conducted to compare subjects with and without the D allele. Data are expressed as means ± SEM. A p value of less than 0.05 was considered significant.

7.3. RESULTS

7.3.1. Subject Characteristics

38 CHF patients were recruited to take part in this study. Baseline characteristics of these subjects are presented in Table 7.2. A χ^2 analysis indicated the genotype frequencies of the CHF patients were in Hardy-Weinberg equilibrium ($p = 0.42$). When separated according to genotype the three groups did not vary significantly in age, height, weight, body mass index (BMI) or left ventricular ejection fraction (LVEF).

Table 7.2. Descriptive Statistics of subjects according to ACE genotype (Mean ± SEM).

	ACE Genotype			
	II	**ID**	**DD**	**P**
N	8	18	12	
Gender (M,F)	8,0	16,2	8,4	
Age (yr)	65 ± 3	63 ± 3	62 ± 4	0.460
Height (m)	1.73 ± 0.03	1.71 ± 0.02	1.69 ± 0.02	0.665
Weight (kg)	75.5 ± 4.0	84.4 ± 3.0	85.4 ± 6.6	0.376
BMI ($kg.m^{-2}$)	25.4 ± 1.5	28.9 ± 1.0	29.9 ± 2.4	0.242
LVEF	29 ± 3	26 ± 2	31 ± 2	0.143
ACE inhibitor	5	16	8	
ARB	1	1	3	

7.3.2. Exercise Tests

There was a strong correlation between peak power attained during the cycle test and $\dot{V}O_{2\ peak}$ ($ml.kg.^{-1}min^{-1}$) in the subjects at baseline ($r = 0.854$; $p < 0.0001$). ANOVA testing revealed a significant association between peak power output and ACE genotype ($p = 0.026$) (Table 7.4). Post Hoc testing revealed a significant difference in peak power ($p = 0.021$) between subjects in the II and DD groups. A trend towards significant differences was also noted between the II and ID genotypes ($p = 0.09$). Despite the strong correlation between peak power output and $\dot{V}O_{2\ peak}$ there was no significant difference between the genotypes in $\dot{V}O_{2\ peak}$ although a t-test revealed a trend towards higher $\dot{V}O_{2\ peak}$ scores in the II compared to the DD genotype ($p = 0.081$). There was no relationship between ACE genotype and quadriceps muscle strength in the CHF patients at baseline.

Table 7.3. Descriptive statistics of subjects who underwent 11-weeks of resistance training (A) or continued normal daily activities (B) according to ACE genotype (Mean ± SEM).

	ACE Genotype			
	II	**ID**	**DD**	**p**
A				
N	4 (21.1%)	10 (52.6%)	5 (26.3%)	
Gender (M,F)	4,0	8,2	4,1	
Age (yr)	66 ± 5	64 ± 4	63 ± 2	0.876
Height (m)	1.75 ± 0.03	1.70 ± 0.03	1.68 ± 0.04	0.602
Weight (kg)	75.4 ± 7.8	86.0 ± 5.0	74.2 ± 2.8	0.250
BMI ($kg.m^{-2}$)	24.7 ± 2.7	29.8 ± 1.7	26.4 ± 1.7	0.217
LVEF	30 ± 4	28 ± 3	27 ± 3	0.868
B				
N	4 (22.2%)	8 (44.4%)	6 (33.3%)	
Gender (M,F)	4,0	8,0	4,2	
Age (yr)	70 ± 3	61 ± 3	67 ± 5	0.310
Height (m)	1.71± 0.04	1.72 ± 0.03	1.70 ± 0.02	0.859
Weight (kg)	75.8 ± 3.9	82.4 ± 2.7	86.2 ± 8.8	0.529
BMI ($kg.m^{-2}$)	26.1 ± 1.6	27.8 ± 0.9	29.8 ± 3.2	0.524
LVEF	27 ± 4	24 ± 2	33 ± 3	0.035

Table 7.4. The effects of ACE genotype on indices of physical performance.

Performance Results	ACE Genotype			ANOVA
	II	**ID**	**DD**	**P**
Peak Power	93 ± 9*	69 ± 7	60 ± 6	0.026
$\dot{V}O_{2\ peak}$	19.4 ± 1.5	16.2 ± 1.3	14.6 ± 1.0	0.097
Quadriceps strength	109 ± 11	113 ± 8	113 ± 9	0.956

Data is presented as mean ± SEM. * $P < 0.05$ for II vs. DD

When subjects were divided into those with the D allele or without, subjects with the D allele had significantly reduced peak power during the incremental exercise test and a lower $\dot{V}O_{2\ peak}$ (Table 7.5). No difference was noted between the groups in baseline quadriceps strength (Table 7.5).

Table 7.5. The effects of the D allele on indices of physical performance

	ACE Genotype		
	II	**ID & DD**	**p**
Peak Power	93 ± 9	66 ± 5	0.026
$\dot{V}O_{2\ peak}$ (ml/kg/min)	19.4 ± 1.5	15.5 ± 0.9	0.049
Quadriceps Strength	112 ± 12	113 ± 6	0.93

Data is presented as mean ± SEM.

7.3.3. Effect of Genotype on Skeletal Muscle Variables.

There were trends towards significant differences in MAPR for four of the five substrate combinations (Table 7.6). When subjects were divided into those with or without the D allele the II group had significantly higher MAPR for the α-KG and PPKM substrate combinations. Similar trends were also observed for the P&M and PC&M substrate combinations (Table 7.7). Since there were only two subjects in the II group these data may be subject to type I error.

Table 7.6. The effects of ACE genotype on skeletal muscle metabolism

Muscle Variables	ACE Genotype			Significance
	II (n = 2)	**ID (n = 9)**	**DD (n = 3)**	**p**
MAPR (μmol/kg/min)				
P&M	7.87 ± 0.86	4.02 ± 0.80	4.23 ± 0.84	0.114
PC&M	3.46 ± 0.47	1.71 ± 0.38	2.49 ± 0.65	0.152
α-KG	6.04 ± 0.03	2.84 ± 0.66	3.51 ± 0.51	0.106
S&R	5.76 ± 2.25	2.43 ± 0.62	2.24 ± 0.23	0.101
PPKM	6.82 ± 0.14	4.46 ± 0.79	4.22 ± 0.71	0.345
Metabolic Enzymes (μmol/kg/min)				
CS activity	17.70 ± 1.61	12.87 ± 1.37	14.49 ± 0.62	0.262
HAD activity	11.43 ± 1.35	12.17 ± 1.35	14.53 ± 0.80	0.702
PFK activity	31.31 ± 4.38	28.07 ± 2.17	26.98 ± 2.12	0.706
LDH activity	45.3 ± 33.5	66.6 ± 11.0	39.2 ± 5.0	0.376

Data is presented as mean ± SEM.

Table 7.7. The effect of the D allele on indices of Muscle Metabolism.

	ACE Genotype		Significance
	II	**ID + DD**	**p**
	(n = 2)	**(n = 12)**	
MAPR (μmol/kg/min)			
P&M	7.87 ± 0.86	4.08 ± 0.61	0.07
PC&M	3.46 ± 0.47	1.90 ± 0.33	0.11
α-KG	6.04 ± 0.03	3.01 ± 0.51	0.0001
S&R	5.76 ± 2.25	2.38 ± 0.46	0.38
PPKM	6.82 ± 0.14	4.40 ± 0.60	0.002
Metabolic Enzymes (μmol/kg/min)			
CS activity	17.70 ± 1.61	13.28 ± 1.04	0.15
HAD activity	31.31 ± 4.38	27.77 ± 1.63	0.59
PFK activity	11.43 ± 1.35	12.81 ± 1.03	0.50
LDH activity	45.29 ± 33.52	59.12 ± 8.79	0.76

Data is presented as mean ± SEM.

7.3.4. Effects of Genotype on Resistance Training Adaptations

Significant differences were noted between genotypes in the response to 11 weeks of resistance training in a subgroup of CHF patients. Subjects with the D allele had greater increases in $\dot{V}O_{2\ peak}$ and peak power output than subjects who were homozygous for the I allele (Table 7.8). Alterations in quadriceps strength were no different between the groups.

Table 7.8. The effects of ACE genotype on skeletal muscle morphology

Muscle Variables	ACE Genotype			Significance
	II	**ID**	**DD**	**P**
	(n = 2)	**(n = 8)**	**(n = 3)**	
Fibre Proportions (%)				
Type I	50.7 ± 8.4	43.4 ± 2.5	51.2 ± 2.2	0.233
Type IIA	18.7 ± 3.6	23.6 ± 3.2	17.9 ± 1.3	0.495
Type IIX	30.7 ± 4.9	33.0 ± 2.2	30.9 ± 3.4	0.831
Fibre Size (μm^2)				
Type I	3006 ± 89	2934 ± 220	3393 ± 258	0.501
Type IIA	3137 ± 272	2894 ± 322	2952 ± 259	0.929
Type IIX	2388 ± 48	2446 ± 244	2023 ± 398	0.605
Capillary/Fibre ratio	1.10 ± 0.12	1.07 ± 0.05	1.12 ± 0.04	0.849
Capillaries (mm^2)	**(n = 2)**	**(n = 8)**	**(n = 2)**	
Type I	1076 ± 15	1140 ± 86	900 ± 56	0.407
Type IIA	1050 ± 109	1026 ± 74	1013 ± 90	0.982
Type IIX	1000 ± 31	1033 ± 99	1361 ± 434	0.436

Data is presented as mean ± SEM.

Table 7.9. Changes in parameters of exercise capacity and muscle strength following 11 weeks of resistance training or continued normal activity.

	ACE Genotype			ANOVA
	II	**ID**	**DD**	**p**
Peak Power				
Trainers	- 13 ± 7	18 ± 3*	18 ± 4*	0.0004
Controls	-3 ± 3	-5 ± 8	-2 ± 5	0.763
$\dot{V}O_{2\ peak}$ **(ml/kg/min)**				
Trainers	-1.9 ± 0.8	1.8 ± 0.8*	1.7 ± 0.7*	0.027
Controls	-2.7 ± 1.0	-2.5 ± 1.4	-1.3 ± 1.5	0.720
Quadriceps Strength				
Trainers	13 ± 9	6 ± 4	20 ± 8	0.261
Controls	-1 ± 6	3 ± 6	-2 ± 7	0.826

Data is presented as mean ± SEM. *P < 0.05 compared to II.

Table 7.10. The effect of the D allele on changes in indices of physical performance as a result of 11 weeks of resistance training.

	ACE Genotype		P Value
	II	ID & DD	
Peak Power	- 13 ± 7	18 ± 2	0.047
$\dot{V}O_{2\ peak}$ (ml/kg/min)	- 1.9 ± 0.8	1.5 ± 0.6	0.007
Quadriceps Strength	13 ± 9	10 ± 4	0.84

Data is presented as mean ± SEM.

7.4. DISCUSSION

7.4.1. Major Findings

The present study demonstrated a relationship between ACE genotype and parameters of endurance exercise performance in CHF patients. $\dot{V}O_{2\ peak}$ and peak power attained during the incremental exercise test were significantly higher in subjects who were homozygous for the I allele than in subjects with the D allele (ID + DD). There was no difference between the groups in quadriceps muscle strength. The second major finding of the present study was that 11 weeks of resistance training exercise resulted in greater improvements in both peak power attained during incremental exercise (Table 7.9) and $\dot{V}O_{2\ peak}$ (Table 7.9) in the ID and DD genotypes compared to the II genotype. However no ACE genotype dependent differences were noted in the strength response to training (Table 7.9).

7.4.2 Baseline Data

7.4.2.1. ACE Genotype and Endurance Exercise

The II genotype is associated with higher $\dot{V}O_{2\ peak}$ and peak power in the present study. These findings are comparable to previous work in healthy subjects (Hagberg et al. 1998; Montgomery et al. 1998), CHF (Abraham et al. 2002) and COPD (Kanazawa et al. 2002) patients. While a relationship between ACE genotype and endurance exercise tolerance has been established, the reasons for the relationship appear less certain.

Recently the I allele has been associated with a greater percentage of type I muscle fibres in human skeletal muscle (Zhang et al. 2003). Such a relationship is likely to be a major factor in the reported association between I allele incidence and endurance exercise tolerance that have

been reported previously (Gayagay et al. 1998; Hagberg et al. 1998; Montgomery et al. 1998). Type I muscle fibres produce ATP via primarily oxidative mechanisms whereas the type II muscle fibres resynthesise ATP with a greater reliance on glycolytic metabolism. It is well known that type I muscle fibres are recruited preferentially during light exercise and that the recruitment of type IIA and then IIX fibres occurs with increasing intensity of exercise (Brooks et al. 2000). All other things being equal, a higher proportion of type I muscle fibres would delay the recruitment of the glycolytic type II fibres, thereby delaying the onset of lactic acidosis which in turn inhibits oxidative metabolism *in vivo* (Conley et al. 2001). In the current study lower $\dot{V}O_{2\,peak}$ and peak power were observed in subjects with the D allele. This study also examined the relationship between skeletal muscle morphology and ACE genotype. Unfortunately no relationship was noted between ACE genotype and type I fibre proportions in the current study although this may well be due to the low subject numbers in the biopsy subgroup (2 II; 8 ID; 3 DD). No significant differences were noted between ACE genotype and indices of muscle oxidative capacity in the current study. When subjects were divided into groups with and without the D allele however, higher MAPR's were observed in two (α-KG and PPKM) of the five substrate combinations examined in patients who were homozygous for the I allele. Trends for similar differences were observed in the P&M and PC&M substrate combinations. Muscle oxidative capacity correlates positively to $\dot{V}O_{2\,peak}$ in CHF patients (Chapter 4). The explanation for a relationship between ACE genotype and muscle oxidative capacity is not clear. Further it must be acknowledged that only 14 CHF patients underwent both ACE genotype and skeletal muscle biopsies and the observations may be affected by the sample size.

Several previous studies have suggested that decreased pulmonary function may have a role in exercise limitation in CHF patients (Mancini 1995; Puri et al. 1995) and that this impaired function may be due to increased neurohumoral activation, in particular that of the RAS system. Previously ACE genotype has been demonstrated to affect the degree of neurohumoral activation associated with CHF (Tiret et al. 1992). Consequently, Abraham et al. (2002) examined the role of ACE genotype on exercise tolerance and lung function in a group of CHF patients. They found an association between the ACE DD genotype and lower $\dot{V}O_{2\,peak}$ in CHF patients. Comparatively lower forced vital capacity, lung diffusion capacity and breathing efficiency (increased $\dot{V}E/\dot{V}CO_2$) were also all noted in DD patients. From these results they concluded that reduced lung volumes and breathing efficiency may negatively impact on endurance exercise capacity in CHF patients. They observed that diffusion impairment was unlikely to be a cause of exercise intolerance in CHF patients as this factor is reduced commensurate with the lower cardiac output, resulting in no change in circulation time in the pulmonary capillaries and no occurrence of arterial oxygen desaturation. Certainly, while diffusion capacity was not measured in the current study, any impairment was insufficient to be a limiting factor as patients remained fully saturated throughout the exercise test. It seems unlikely therefore that impaired pulmonary diffusion capacity is the cause of exercise intolerance in CHF patients. Increasing the work of breathing has been demonstrated to have deleterious effects on non respiratory muscle blood flow during maximal exercise while having no effect on total blood flow (Harms et al. 1998). This should not affect $\dot{V}O_{2\,peak}$ as the non respiratory muscle $\dot{V}O_2$ and blood flow would be offset by the increase in respiratory muscle $\dot{V}O_2$ and blood flow. However, the peak power output may be affected by such a phenomenon.

Indeed, Harms et al. (1998) reported no change in $\dot{V}O_2$ despite decreases in skeletal muscle blood flow at maximal exercise.

Reduced muscle blood flow and oxygen extraction have also been suggested as potential explanations for the differences in $\dot{V}O_{2\,peak}$ between genotypes. Kanazawa et al. (2002) investigated 39 Japanese patients with COPD and reported an association between the ACE DD genotype and an impairment in peripheral tissue oxygenation during exercise, consistent with impaired diffusion of oxygen across the muscle microcirculation. These findings are consistent with those of Hagberg et al. (1998) who observed a greater maximal a-VO_2 difference with higher $\dot{V}O_{2\,peak}$ in post-menopausal women of II genotype. Cardiac output was not different in these subjects, meaning the difference was entirely due to differences in oxygen extraction by the muscle.

There are a number of possible ways in which muscle oxygen extraction may be affected by ACE genotype. ACE II genotype has previously been associated with prolonged bradykinin half-life (Murphey et al. 2000) and reduced production of angiotensin II (Brown et al. 1998). These effects would be expected to result in enhanced vasodilatation causing reductions in peripheral vascular tone and consequently increased blood flow through the capillaries, thereby favouring substrate delivery to resting muscles at least. It is unclear however whether such a mechanism would increase blood flow to exercising muscles where muscle blood flow is largely if not exclusively under metabolite control (Delp and Laughlin 1998). In fact it has been demonstrated that decreased production of angiotensin II will have little if any effect on muscle blood flow at maximal exercise (Symons et al. 1999). In addition, Danser et al. (1999) have

previously reported the fractional conversion of infused angiotension I to angiotensin II in the forearm of healthy human subjects to be similar across the different genotypes. In addition they reported no genotype related differences in the plasma angiotensin II to angiotensin I ratio, suggesting that effects other than enhanced conversion underlie the reported associations between the D allele and cardiovascular disease.

A secondary function of the ACE gene is its role as a mediator of bradykinin degradation (Murphey et al. 2000). Murphey et al. (2000) infused bradykinin into the brachial artery of nine volunteers of each ACE genotype and reported that the D allele was associated with a higher rate of bradykinin degradation. Bradykinin exerts a vasodilatory effect on the vasculature in a dose dependent manner (Tom et al. 2002). The association between the higher rate of bradykinin degradation and incidence of the D allele may result in reduced bradykinin induced vasodilation in maximal exercise in subjects with the D allele. More recently it has been demonstrated that the concentration of bradykinin rises in skeletal muscle during exercise (Langberg et al. 2002). This result provides a possible mechanism for the role of bradykinin in exercise induced hyperaemia in skeletal muscle and may provide a possible explanation for reportedly lower $\dot{V}O_{2\ peak}$ in subjects with the D allele.

Another possible explanation for the reported lower $\dot{V}O_{2\ peak}$ in patients with the D allele may be the gender distribution of subjects in the present study. Due to smaller body sizes, females generally obtain lower $\dot{V}O_{2\ peak}$ (L/min) than their male counterparts. Even when these results are expressed as a function of body weight (ml/kg/min) females have lower values of $\dot{V}O_{2\ peak}$ due to their higher body fat percentages. The present study investigated the relationship between

ACE genotype and parameters of physical fitness in 32 male and six female CHF patients. There were no females in the group homozygous for the II allele. When the analyses were conducted on the male patients alone (Appendix IV) there was still a tendency for lower $\dot{V}O_{2\ peak}$ ($p= 0.14$) and peak power ($p = 0.07$) in subjects with the D allele. Consequently it is still probable that in CHF patients there is an ACE genotype effect on indices of aerobic exercise performance. The results of the current study can not be attributed to ethnicity as all patients were Caucasian and more than 90% were of Anglo-Saxon origin.

It is clear that CHF patients homozygous for the I allele have higher indices of aerobic power than other patients. The mechanism for this difference however remains to be elucidated but differences in muscle oxidative capacity linked to elevated proportions of oxidative (type I) muscle fibres provide at least one possible explanation.

7.4.2.2. ACE Genotype and Strength

Myerson et al. (1999) studied 91 British Olympic standard runners and reported I allele frequency to increase linearly with event distance. They concluded from their results that the I allele may be important in endurance based sports and the D allele may be important in power or strength based sports. It should be noted however that this observation is based upon a correlation and does not provide an underlying mechanism. By contrast however, the current study found no relationship between the D allele and muscle strength at baseline testing. Muscular strength is influenced by factors such as neural stimulation, muscle fibre proportions and muscle fibre size. It is not apparent how these variables may be affected by ACE genotype. The data reported here represent the first analyses of any potential relationship between the ACE

genotype and skeletal muscle variables known to be related to muscle strength. No ACE genotype related differences were found in either muscle fibre type proportions or fibre sizes. This supports the contention that ACE genotype is not directly related to muscle size or strength although it must be acknowledged that the lack of any ACE genotype related difference may be due to the small sample size.

7.4.3. Effect of ACE genotype on Responses to Exercise Training

ACE genotype was found to have a significant effect on changes to $\dot{V}O_{2\ peak}$ (Table 7.9) and peak power (Table 7.9) as a result of 11 weeks of low intensity resistance training in a subgroup of CHF patients. Subjects with the D allele made significantly greater improvements in both peak power and $\dot{V}O_{2\ peak}$ than subjects who were homozygous for the I allele.

There are a number of factors that may be involved in the prognosis of patients with CHF. Many of these factors act in a feed forward manner, which is that a particular factor in the disease state will stimulate a response, the end result of which will be to increase the initial factor. A number of mechanisms in the progression of CHF can be described in this manner and have been discussed in section 2.6. One possible mechanism is the role of hormonal activation in increasing peripheral resistance, which then results in worsening ventricular function and further hormonal activation (Figure 2.9). A major hypothesis for the lower baseline $\dot{V}O_{2\ peak}$ in subjects with the D allele is that of increased peripheral resistance. Increased peripheral resistance in the early stages of CHF may result in greater ventricular dysfunction and consequently greater peripheral resistance further reducing substrate delivery to the exercising muscles. Exercise training has been demonstrated to reduce neurohormonal (noradrenaline) control of the vasculature in CHF patients (Tyni-Lenne et al. 2001). It is possible that subjects

with the D allele may be subject to a higher degree of hormonally induced increases in peripheral resistance. Training that reduces the extent of neurohormonal control mechanisms may therefore be of greater benefit to these subjects.

Changes in the regulation of bradykinin may be involved in the greater increase in $\dot{V}O_{2\ peak}$ that was observed following training in the subjects with the D allele than in those who are homozygous for the I allele. The rate of degradation of bradykinin is at least partially dependent on ACE genotype with D allele incidence being linked to a higher rate of bradykinin degradation (Murphey et al. 2000). It is possible, although currently not demonstrated, that because of greater ACE activities, bradykinin concentration is reduced in subjects with the D allele thereby limiting its vasodilatory effects at rest. The difference in circulating bradykinin concentration may be exacerbated during exercise when bradykinin levels in the peripheral vasculature are elevated (Langberg et al. 2002). Chronic exercise training results in increases in the sensitivity to bradykinin in the brachial arteries of swine *in vitro* (McAllister and Laughlin 1997; Laughlin et al. 2001). If this phenomenon were to occur in human vasculature in response to exercise training it may at least partially account for the difference in endurance improvement noted in subjects with the D allele compared to those homozygous for the I allele. An increase in bradykinin sensitivity could potentially benefit subjects with the D allele to a greater extent than the II group because of their lower bradykinin concentrations. The functional result the higher bradykinin after training and subsequent vasodilatation would be an increased skeletal muscle perfusion during exercise, and hence a delayed onset of lactic acidosis and fatigue. The effect of training on lactic acidosis was described in chapter 5 with CHF patients displaying elevated peak lactate during exercise following training compared to baseline results. This result in itself

may be indicative of increased muscle blood flow allowing a greater flux of lactate from the cells into the bloodstream for full removal.

Alternatively, it is possible that the greater response to training reported in subjects with the D allele was due to lower baseline results in these subjects. $\dot{V}O_{2\ peak}$ was approximately 15% lower at baseline in subjects with the D allele compared to those who were homozygous for the I allele. Subjects performed a range of resistance exercises for around 30 seconds and then rested until their heart rate had returned to within 10 beats per minute of resting values. At the onset of the study it was believed that the intensity would be sufficient to stimulate a training response (including an aerobic response) in the CHF patients due to their low initial exercise tolerance. While the results of subjects with the D allele indicate that this was indeed the case in these subjects, the training stimulus may have been insufficient in subjects homozygous for the I allele due to their higher baseline aerobic fitness. This explanation appears unlikely to have had any major influence on the results of the current study however, as there was little relationship between baseline $\dot{V}O_{2\ peak}$ and the change in $\dot{V}O_{2\ peak}$ following the training intervention ($r = 0.36$; $p = 0.129$).

7.4.4. Study Limitations.

A major limitation of this study was the small number of subjects measured for ACE genotype particularly in the muscle biopsy group. This prevented any analysis of the effects of ACE genotype on the response to resistance training. Consequently, despite the use of statistical analyses, the results of this study must be treated as observational and preliminary. Trends towards significantly lower aerobic exercise capacity in patients with the D allele compared to

those without have been reported. However it is important to note that only seven subjects were homozygous for the I allele meaning that no conclusive comparison could be made. If this result were to continue in a larger sample population it may be possible to make conclusive statements about the role of ACE genotype in exercise tolerance in CHF.

Pharmacological agents designed to control ACE activity are in common use in CHF. The patients who were subjects in the current study were taking either ACE inhibitors (ACEi) or Angiotensin II receptor blockading (ARB) drugs. These drugs operate via a number of mechanisms but have a common result of reducing peripheral resistance. While all patients in this study were taking either ACEi or ARB drugs (Table 7.1) the doses of these pharmacological agents were not controlled for. Consequently these and other medications may have influenced the results obtained.

7.5. CONCLUSION

The major finding of this study was that CHF patients homozygous for the I allele had a higher $\dot{V}O_{2\ peak}$ and peak power during incremental exercise than patients with the D allele. This was associated with a higher level of muscle oxidative capacity as determined by MAPR. Somewhat paradoxically patients with the D allele displayed greater increases in $\dot{V}O_{2\ peak}$ as a result of 11 weeks of resistance training. Whilst it is acknowledged that the current study is limited by the small sample size, the relationships that have been identified provide clear support for further investigations using larger subject numbers.

CHAPTER 8. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

8.1. SUMMARY

This thesis comprises four studies. The first study examined skeletal muscle fibre type proportions, size and capillarity, mitochondrial ATP production rate (MAPR), and the activities of a range of oxidative and glycolytic enzymes *in vitro* in a group of chronic heart failure (CHF) patients and a group of healthy similarly aged control subjects. A battery of exercise tests was also used to compare exercise performance in these subjects. The second study determined the effects of 11 weeks of low intensity resistance training on exercise performance (muscle strength and $\dot{V}O_{2\ peak}$) and skeletal muscle fibre type proportions, size and capillarity, MAPR, and oxidative and glycolytic enzyme activities in a group of CHF patients. Study three examined the role of skeletal muscle nitric oxide synthase (NOS), circulating TNF-α concentrations and circulating cortisol and testosterone ratios as possible mediators of skeletal muscle adaptations to CHF and resistance training. The final study examined the role of ACE genotype on exercise tolerance in CHF and the response of CHF patients to resistance training. The major findings of these studies are summarised below.

8.1.1. Major Findings in the Study Comparing CHF patients and Healthy Controls.

Chapter 4 investigated the role of skeletal muscle parameters on exercise tolerance in CHF patients compared to healthy similarly aged control subjects. The major findings of this study were:

- CHF patients showed a significantly lower exercise performance indicated by a lower peak work rate, $\dot{V}O_{2\ peak}$ and lactate threshold than the control subjects. Muscle strength in these patients was also significantly lower than the control subjects.
- There was no difference in any of the indices of skeletal muscle oxidative capacity measured between the CHF patients and control subjects despite the large differences observed in exercise tolerance.
- Type I muscle fibres are smaller in CHF patients compared to healthy control subjects.

The smaller size of the type I muscle fibres and the lower lean thigh mass to body mass ratio in the CHF patients provide a likely mechanism for the lower exercise tolerance in this group. The reduction in the cross-sectional area of the type I muscle fibres suggests that CHF patients may recruit type II muscle fibres at lower exercise intensities. This would be expected to result in an earlier onset of lactic acidosis, which was observed in this study. While there was no difference in muscle oxidative capacity between the CHF patients and healthy control subjects, *in vivo* oxidative phosphorylation can not be discounted as a further mechanism for the low exercise tolerance in CHF patients. Larger glycolytic fibre proportions in CHF patients may reduce the sensitivity of muscle oxidative phosphorylation to ADP stimulation and thereby contribute to the exercise intolerance noted in this study. An earlier onset of lactic acidosis may also contribute to a reduced activation of oxidative phosphorylation during exercise.

8.1.2. Major Findings in the Resistance Training Study.

Chapter 5 was the first study to comprehensively examine the effects of a resistance training protocol on skeletal muscle in CHF patients. The major findings in this study were that:

While there were no significant differences between the subjects randomised to the training or control groups prior to the intervention, exercise tolerance as determined by $\dot{V}O_{2\,peak}$ had increased following the intervention in the training group and decreased in the control group. Similarly thigh muscle peak torque was increased in the resistance training group at a velocity of $60^{\circ}\ sec^{-1}$ as measured by a MERAC dynamometer.

MAPR as indicated by the substrate combination P+M was significantly increased in the training group following the resistance training program. Additionally, MAPR was reduced in the same substrate combination following the intervention in the control subjects. These results were further supported by results obtained from the analysis of the oxidative enzymes CS and HAD both of which were increased in the training group following the 11 week intervention.

No difference was observed in the proportions of skeletal muscle type I, IIA or IIX or in the cross-sectional areas of these fibres between the groups at either baseline or endpoint. This may be due to the intensity of the resistance training protocol being insufficient to stimulate muscle fibre hypertrophy. Consequently the improvements in exercise tolerance reported in CHF patients following eleven weeks of resistance training are likely due to improvements in muscle oxidative function as a consequence of improved muscle oxidative capacity and to improved neuromuscular facilitation as a result of the training program.

8.1.3. Major Findings in the Chemical Messenger Study.

Chapter 6 examined the role of a variety of chemical messenger molecules on the exercise intolerance and muscle parameters in CHF. The effect of eleven weeks of resistance training by

CHF patients on the concentration or expression of these molecules was also investigated. The major finding of this study was that there was a trend towards a higher circulating TNF-α concentration in the CHF patients compared to healthy control subjects at baseline. Despite well established pathways by which TNF-α may cause exercise intolerance through its effects on skeletal muscle (Anker et al. 1997a; Reid and Li 2001; Fernandez-Celemin et al. 2002) no relationship was noted between TNF-α and any measurement of either exercise tolerance or muscle metabolism or morphology. Two separate pathways leading to reductions in exercise tolerance are likely to exist in CHF. The initial decreases in $\dot{V}O_{2\ peak}$ occur early in heart failure and may not involve alterations in skeletal muscle wasting. It is unlikely that increases in circulating TNF-α concentration will have a role in this pathway. The second pathway involves additional decrements in $\dot{V}O_{2\ peak}$ due to alterations in muscle morphology. It appears likely that TNF-α has a major role in this pathway. In addition, no differences were noted between CHF patients and healthy control subjects in any of the other molecules measured and resistance training did not result in changes to any of the signalling molecules investigated. Many of the findings in the current study are discordant with those widely reported in the literature (Levine et al. 1990; Anker et al. 1997a; Anker et al. 1997b; Cicoira et al. 2001a; Greiwe et al. 2001) and are likely due to a small sample size and the clinical status of the CHF patients recruited in the study. It must be noted that while TNF-α has a role in the muscle wasting and eventual cachexia that are associated with exercise intolerance in CHF, there appear to be other mechanisms not involving the chemical messengers investigated in chapter 6 that also play a significant role in the exercise intolerance.

8.1.4. Major Findings in the ACE Gene Study

Chapter 7 examined the role of ACE gene polymorphism on exercise tolerance and a range of muscle parameters in CHF. Further to this, the effect of ACE genotype on responses to an eleven week resistance training protocol was also examined. The major findings in this study were:

- $\dot{V}O_{2\,peak}$ and peak power output during incremental exercise were higher in patients who were homozygous for the I allele than in patients in either the ID or DD genotype. However there were no genotype related strength differences.
- Eleven weeks of resistance training caused increases in $\dot{V}O_{2\,peak}$ and peak power obtained during incremental exercise in patients with the D allele but not those homozygous for the I allele.
- Significant higher levels of MAPR (in the presence of α-KG and PPKM) were observed in subjects who were homozygous for the I allele. Similar trends were observed for MAPR in the presence of P&M and PC&M. No differences were observed between the genotypes for any of the other muscle parameters.

The higher $\dot{V}O_{2\,peak}$ in patients homozygous for the I allele indicates the potential role for ACE genotype as a regulatory factor in exercise performance. Potential mechanisms by which ACE genotype may influence endurance exercise capacity include a potential role in muscle fibre type proportions, muscle oxidative capacity and control of muscle blood flow during exercise through the regulation of circulating bradykinin levels. While no relationship was noted between muscle fibre proportions and ACE genotype in the subjects used in this thesis it is possible that this is due to the small numbers of subjects with the II and DD genotypes. The improvement in

$\dot{V}O_{2\,peak}$ and peak power noted in the ID and DD but not the II genotypes following the resistance training intervention is likely due to better vasoactive control by bradykinin in the ID and DD genotypes although higher baseline $\dot{V}O_{2\,peak}$ results in the patients homozygous for the I allele can not be discounted as a potential factor in the lack of improvement in this group.

8.2. RECOMMENDATIONS FOR FURTHER RESEARCH

While it is accepted that skeletal muscle oxidative function is impaired in CHF patients the exact cause of the impairment requires elucidation. The issues raised from this thesis indicate that skeletal muscle size, morphology and oxidative function *in vivo* are likely causes of impaired exercise tolerance in CHF patients. This thesis also demonstrated that a resistance training protocol can result in improvements in exercise tolerance and muscle oxidative capacity in these patients. The mechanisms behind these adaptations however require further investigation as mediators of muscle blood flow (iNOS and nNOS) and anabolic/catabolic function (TNF-α, cortisol, testosterone) were no different between CHF patients and controls and did not alter with training.

Accordingly, the recommendations for further investigation are listed below:

- A modified method of the MAPR assay utilising variable concentrations of ADP and creatine should be employed to investigate possible causes of impaired muscle oxidative function in CHF patients. This study may reveal whether alterations in the sensitivity of metabolic processes to these metabolites contribute to reductions in oxidative function *in vivo* that are not observed under ideal conditions.

- An expanded resistance training protocol utilising a longer training duration and the adoption of a training stimulus incorporating an eccentric component should be investigated in CHF patients. The findings of this thesis were that concentric resistance training results in improvements in muscle oxidative capacity but no alterations in muscle structure in CHF patients. Resistance training incorporating an eccentric component has previously been demonstrated to result in greater muscle adaptations than concentric training alone. As muscle atrophy appears to be a major factor in the exercise intolerance experienced in CHF, resistance training incorporating an eccentric component may be of significant benefit to these patients.
- A large scale study examining the effect of ACE genotype on response to both endurance and resistance training in CHF should be undertaken. The results obtained using a small number of subjects was reported in Chapter 7 however much larger numbers of subjects are required before any concrete conclusions regarding the suitability of different genotypes for a particular exercise stimulus can be made.

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APPENDIX I. DAILY RESISTANCE TRAINING DIARY

TRAINING WEEK ☐ **DAY** ☐ **FILE NUMBER** ☐☐☐☐☐☐ **DATE** ☐☐/☐☐/☐☐

1. Has the volunteer experienced any problems since the last visit? YES / NO (If YES, please complete an Adverse Event Sheet.)
2. Time of last meal.....
3. **EXERCISE STRESS TEST: PEAK HR**.....**PEAK BP**.....
4. PRE-EXERCISE: HR.....BP.....

			CIRCUIT 1				CIRCUIT 2			
Changes	**Activity**	**Work Load**	**Reps**	**Peak HR**	**Recovery HR**	**Recovery Time**	**Reps**	**Peak HR**	**Recovery HR**	**Recovery Time**
	Warm-up	5 min								
	Bike	min / watts								
	Chest Press	Setting:								
	Walk	.....min								
	Arm cycle	min / revs								
	Leg extension	Setting:								
	Shoulder Press	Setting:								
	Bike	min / watts								
	Cool Down	5 min								

APPENDIX II. DESCRIPTIVE STATISTICS OF TRAINING STUDY PATIENTS

	Exercise group (n=18)		Control group (n=23)	
	Entry	Exit	Entry	Exit
Age, yrs	65 ± 10		65 ± 9	
Male / Female	15 / 3		21 / 2	
Height (cm)	170 ± 9		171 ± 7	
LVEF%	28 ± 7		28 ± 7	
NYHA	2.3 ± 0.5		2.3 ± 0.4	
Weight (kg)	80.3 ± 14.8	81.7 ± 15.7	80.2 ± 13.2	82.2 ± 12.7
CHF diagnosis				
Ischemic heart disease	11		16	
Dilated cardiomyopathy	7		7	
Medications				
Angiotensin converting enzyme inhibitor or angiotensin receptor blocker	18 (100%)	18 (100%)	23 (100%)	22 (96%)
Diuretic	18 (100%)	18 (100%)	20 (87%)	20 (87%)
Beta-blocker	10 (56%)	10 (56%)	13 (57%)	12 (52%)
Digoxin	11 (61%)	11 (61%)	14 (61%)	14 (61%)
Aspirin	6 (33%)	6 (33%)	10 (43%)	13 (57%)
Warfarin	11 (61%)	12 (67%)	13 (57%)	12 (52%)
Amiodarone	17 (94%)	14 (78%)	18 (78%)	19 (83%)
Nitrates	18 (100%)	18 (100%)	23 (100%)	23 (100%)
Calcium channel antagonist	17 (94%)	17 (94%)	21 (91%)	21 (91%)

Descriptive characteristics of the 41 heart failure patients who underwent the series of tests comprising skeletal muscle strength and endurance and symptom-limited graded exercise test for the assessment of aerobic power ($\dot{V}O_{2peak}$). Data presented as mean ± SD.

APPENDIX III. TABLES OF CORRELATIONS BETWEEN MECHANISTIC VARIABLES AND EXERCISE AND SKELETAL MUSCLE PARAMETERS

	TNF-α	Cortisol	Testosterone	C:T Ratio	nNOS	iNOS
TNF-α		- 0.026	- 0.088	- 0.029	- 0.153	- 0.467
Cortisol			- 0.041	0.905*	0.641*	- 0.148
Testosterone				- 0.451	- 0.177	0.384
C:T Ratio					0.616*	- 0.279
nNOS						0.387
iNOS						

Table 1. The relationship between mechanistic variables in CHF patients. *Denotes $p < 0.05$.

	TNF-α	Cortisol	Testosterone	C:T Ratio	nNOS	iNOS
$\dot{V}O_{2\ peak}$	- 0.374	0.022	0.583*	- 0.323	- 0.131	- 0.133
MAPR						
P+M	- 0.064	- 0.448	0.347	- 0.569	- 0.187	- 0.053
PC+M	- 0.313	- 0.362	0.335	- 0.444	- 0.202	0.209
α-KG	- 0.219	- 0.358	0.385	- 0.481	- 0.079	0.322
S+R	- 0.032	- 0.118	0.281	- 0.199	0.036	0.062
PPKM	- 0.321	0.089	0.250	0	- 0.108	- 0.274
CS	- 0.137	- 0.097	0.475	- 0.284	0.016	0.349
HAD	0.427	0.049	0.024	0.026	- 0.332	- 0.151
PFK	0.309	0.193	- 0.048	- 0.124	0.019	- 0.209
LDH	- 0.280	- 0.445	- 0.240	- 0.307	0.498*	- 0.121

Table 2. Relationship between metabolic and mechanistic variables. *Denotes $p < 0.05$.

	TNF-α	Cortisol	Testosterone	C:T Ratio	nNOS	iNOS
Strength	- 0 .258	0.428	0.216	0.282	0.073	- 0.398
Muscle Fibre Proportions						
Type I (%)	0.041	0.204	0.101	0.404	- 0.273	- 0.349
Type IIA (%)	0.252	0.014	- 0.142	- 0.190	0.235	0.108
Type IIX (%)	- 0.299	- 0.221	0.026	- 0.030	0.098	0.333
Muscle Fibre Size (μm^2)						
Type I	- 0.490	0.246	0.311	0.052	0.419	- 0.129
Type IIA	- 0.041	0.386	0.273	0.190	0.336	0.039
Type IIX	- 0.275	0.476	0.176	0.362	0.101	0.149
Capillary to Fibre Ratio	0.278	0.002	0.665*	- 0.383	0.313	- 0.149

Table 3. Relationship between morphologic and mechanistic variables. *Denotes $p < 0.05$.

APPENDIX IV. EXERCISE DATA FOR MALE CHF PATIENTS ENROLLED IN THE ACE GENOTYPE STUDY

Performance Results	ACE Genotype			ANOVA
	II	**ID**	**DD**	**P**
Peak Power	93 ± 9	75 ± 6	65 ± 6	0.08
$\dot{V}O_{2\ peak}$	19.4 ± 1.5	16.7 ± 1.4	16.3 ± 1.0	0.36
Quadriceps strength	109 ± 11	116 ± 9	122 ± 12	0.18

ACE genotype and indices of physical performance in male CHF patients. Data presented as mean ± SEM.