SKELETAL MUSCLE MITOCHONDRIAL CAPACITY AND METABOLISM IN LUNG TRANSPLANT PATIENTS AND RESISTANCE TRAINED SUBJECTS

By

XIAO NAN WANG (MD)

July 2000

Dissertation submitted for the degree of

Doctor of Philosophy

Supervisor:

Associate Professor Michael F. Carey

Department of life Sciences and Technology

and

Co-supervisor Dr. Michael J. McKenna

Department of Human Movement, Recreation and Performance,

Victoria University of Technology,

Victoria, Australia.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I wish to particularly offer my warmest and most sincere thanks to Associate Professor Michael Carey, my tutor and supervisor, for without his support, extensive knowledge of physiology and biochemistry, his encouragement and his invaluable guidance in the preparation of this manuscript, I could not have learned from what has been most enjoyable years of study.

I wish to thank Dr. Michael McKenna, for his co-supervision, and encouragement throughout my Ph.D. study. I am also grateful to Professor David Lawson, Dean of the Faculty of Human Development and Professor Terry Roberts for their support during my candidature and for giving me the opportunity to undertake this study in Victoria University. I am also like to thank Professor Robert Fairclough for the opportunity to undertake these studies in the School of Life Sciences and Technology.

Special mention must be to made to my schoolmates at university Dr. J. L. LI, and Mr. S. F. Fraser, who were always willing to help me and provide support throughout the years.

I wish to acknowledge the support of Dr Trevor Williams, Dr Judy Morton and Ms E. Side and Dr. G. Snell from the Department of Respiratory Medicine, Alfred Hospital and Monash Medical School, Melbourne. Particularly to Dr. Trevor Williams for his excellent contribution in lung transplantation and cyclosporine studies.

I wish to acknowledge assistance by Mr. Tim Wrigley in the muscle isokinetic measurements.

I would also like to thank, Ms. Andrea Watson, for her help in preparing this manuscript.

Finally, I wish to acknowledge and thank the people whose comments, support, and enthusiasm encouraged me to improve the manuscript. I would also like to thank all of the subjects who participated in these studies.

Publication and presentations arising from this work

JOURNAL PUBLICATION

Wang, X. N., T. J. Williams, M. J. McKenna, J. L. Li, S. F. Fraser, E. A. Side, G. I. Snell, E. H. Walters, and Michael F. Carey. (1999). Mitochondrial capacity, muscle fibres and metabolism in skeletal muscle post lung transplant. *Am. J. Respir. Crit. Care Med.* 160: 57-63.

CONFERENCE PRESENTATIONS

Carey, M. F., X. N. Wang, M. J. McKenna, J. L. Li, S. F. Fraser, E. A. Side, G. I. Snell, E. H. Walters, and T. J. Williams. (1996). Resting skeletal muscle metabolism and metabolic enzymes in lung transplant recipients. *First International Conference on Exercise Science, Griffith University, Gold Coast QLD, Australia.* (11-14 July): Section 2:

Wang, X. N., M. F. Carey, M. J. McKenna, J. L. Li, S. F. Fraser, E. A. Side, G. I. Snell, E. H. Walters, and T. J. Williams. (1996). Skeletal muscle fibre types and muscle mitochondrial ATP production rates post lung transplant. *First International Conference on Exercise Science, Griffith University, Gold Coast QLD, Australia.* (11-14 July): Section 10.

Williams, T. J., X. N. Wang, M. J. McKenna, J. L. Li, S. F. Fraser, E. A. Side, G. I. Snell, E. H. Walters, M. F. Carey. (1996). Low mitochondrial ATP production rate in human skeletal muscle post lung transplant. *Am. J. Respir. Crit. Care Med. 153 A.*

Wang, X. N., J. L. Li, M. J. McKenna, T. J. Williams, & M. F. Carey. (1997). Cyclosporine reduces *in vitro* mitochondrial ATP production rate in rat skeletal muscle. *The Transplantation society of Australia and New Zealand Fifteenth Annual Scientific Meeting. 33P.*

Wang, X. N., J. L. Li, M. J. McKenna, S. F. Fraser, G. I. Snell, T. J. Williams, and M. F. Carey. (1997). Skeletal muscle metabolic enzymes and fibre type alteration post lung transplant. *The Transplantation society of Australia and New Zealand Fifteenth Annual*

Scientific Meeting. 33P.

Wang, X. N., M. J. McKenna, J. L. Li, S. F. Fraser, G. I. Snell, M. F. Carey, & T. J. Williams. (1997). Abnormal skeletal muscle substrates and metabolites in lung transplant recipients. *The Transplantation Society of Australia and New Zealand Fifteenth Annual Scientific Meeting. 33P.*

DECLARATION

This dissertation summarises original work conducted in the Department of Life Science and Technology and the Department of Human Movement, Recreation and Performance, at Victoria University of Technology.

This thesis is the result of work performed by the author. However, due to the complexity of the studies and the invasive procedures undertaken on subjects during the exercise tests, collaboration for this research was considerable. Medical personnel conducted the muscle biopsies. Mr. Steve Fraser assisted with the organisation of subjects for the experimental trials and in conducting the exercise tests. Associate Professor Michael Carey, Dr. Michael McKenna, and Dr. Steve Selig helped in conducting the exercise tests. Dr. Jia Li Li assisted with part of work conducted in the Exercise Metabolism Unit Laboratory and in conducting the exercise tests.

Xiao Nan Wang

ABSTRACT

Lung transplant (LTx) recipients have poor exercise tolerance, which persists in spite of the restoration of near normal lung function. This suggests that the exercise limitation is related to defects in skeletal muscle. In Study I (Chapter 4), mitochondrial function and metabolism in resting skeletal muscle were examined for 7 LTx recipients, 3-24 months post operation. Exercise performance for patients with lung transplantation was also investigated. The results demonstrated that the average $\dot{V}O_2$ peak, peak workrate and the average duration of exercise in the LTx patients was ~51%, 41% and ~60% of the control level and ($P < 0.05$), respectively. The lactate threshold (LT), determined during incremental exercise tests, was significantly lower in the LTx patients ($P < 0.05$). Compared with the controls, the skeletal muscle in LTx recipients exhibited:

(1) lower proportion of type I ($P < 0.01$).

(2) higher lactate ($P < 0.01$) and higher inosine monophosphate (IMP) ($P < 0.01$) and lower adenosine triphosphate (ATP) ($P < 0.01$) contents at rest;

(3) lower activity of mitochondrial enzymes (GDH, CS, OGDH and HAD; $P < 0.005$), but no difference in the activity of glycolytic and glycogenolytic enzymes (HK, PK and PHOSPH), except for phosphofructokinase (PFK) which was higher ($P < 0.05$);

(4) lower mitochondrial ATP production rate (MAPR) with all substrate combinations ($P < 0.05$) including pyruvate + malate (P + M), palmitoyl-L-carnitine + malate (PC + M), succinate + rotenone (S + R), α-ketoglutarate (α-KG) and pyruvate + palmitoyl-L-carnitine + α-ketoglutarate + malate (PPKM).

These differences demonstrate that LTx patients have a low oxidative and mitochondrial capacity in skeletal muscle. These results also indicate that skeletal muscles in these patients have a high reliance on the anaerobic metabolism at rest and during exercise. Accordingly, these abnormalities may play important roles in the limitation of exercise capacity in LTx

patients.

Cyclosporine A (CyA) has been considered as a factor impairing skeletal muscle function in organ transplant recipients. To determine the effect of CyA on oxidative capacity in skeletal muscle, three concentrations of CyA, 50 μg ml^{-1}, 25 μg ml^{-1}, and 1 μg ml^{-1}, were employed to examine the effect of CyA on mitochondrial ATP production rate (MAPR) *in vitro* in Study II (Chapter 5). In this study, 8 female Sprague-Dawley rats were sacrificed and the red gastrocnemius muscle used for isolation of mitochondria. Four groups of substrates were used as P + M, PC + M, S + R and PPKM. MAPR in the presence of all groups of substrates was significantly inhibited by 50 μg ml^{-1}, 25 μg ml^{-1}, and 1 μg ml^{-1} concentration of CyA ($P < 0.05$). The extents of decline in MAPR with various substrates were similar. These findings indicate that CyA could be a factor impairing mitochondrial function in skeletal muscle even at low concentrations, similar to those be found in lung transplantation recipients.

Resistance training is an effective exercise mode for improving muscle bulk and strength in sports and medical rehabilitation. Recently, resistance training has become a popular exercise mode to improve muscular function and enhance exercise performance in patients with cardiopulmonary diseases. In Study III (Chapter 6), sixteen male volunteers participated in a study on the effects of resistance training on mitochondrial oxidative capacity in skeletal muscle. The subjects included eight untrained controls (UT) and eight long-term resistance-trained subjects (RT). The results of this study exhibited that long-term resistance-trained subjects have a significantly larger thigh cross-sectional area ($P < 0.01$) and lower skinfold thickness in the thigh of the RT subjects ($P < 0.05$) than the UT controls. During knee extension exercise, the levels of leg muscle peak torque at angular velocities of 60° to 360° sec^{-1} were significantly higher in the RT compared with the controls ($P < 0.05$). The levels of leg muscle peak torque at angular velocities of 60° to 360° sec^{-1} were significantly correlated

with the CSA of thigh muscle ($P < 0.05$). Fatigue index determined during 50 consecutive knee extensions, however, was not different in resistance trained subjects compared with controls. Glycolytic enzymes HK and PK activities expressed per unit muscle weight were significantly higher in RT group than the UT group ($P < 0.05$). The resting muscles in the RT subjects contained higher amounts of PCr and TCr than the UT controls ($P < 0.05$) indicating the resistance training increased phosphagen storage in muscle. During the intensive leg exercise the concentrations of ATP, PCr and glycogen were significantly reduced in fatigued muscle compared with the resting muscles in both the groups ($P < 0.05$). In contrast, the IMP, Cr and lactate significantly accumulated in fatigued muscle in both the groups ($P < 0.05$). The muscle metabolites were not different in fatigued muscles between the two groups. The muscle fibre type proportions, oxidative enzyme activities and MAPR in present of various substrates in the resistance-trained subjects were not significantly different compared with the controls. Consequently, resistance trained subjects have a higher total mitochondrial capacity in skeletal muscle because the RT subjects have a larger thigh muscle mass compared with the controls. This form of training, therefore, may be useful in the rehabilitation for the patients with heart and lung diseases.

ABBREVIATIONS

α-KG	α-ketoglutarate or 2-oxoglutarate
[Ca2$^+$]	Ca^{2+} concentration
$^{\bullet}$OH	hydroxyl radical
A_1TD	α_1-antitrypsion deficiency.
Acetyl-CoA	acetyl-coenzyme A
ADP	adenosine diphosphate
AK	adenylate kinase
AMP	adenosine monophosphate
AMRs	ATP monitoring reagent solution
ATP	adenosine triphosphate
AZA	azathioprine
BF	body fat
BLTx	bilateral lung transplantation
Cat	catalase
CF	cystic fibrosis
CHD	congenital heart diseases
CHF	congestive heart failure
CI	cardiac output/body surface area
COPD	chronic pulmonary obstructive diseases
CPK	creatine phosphokinase
CPT	carnitine palmitoyltransferase
CPTI	carnitine palmitoyltransferase I
CPTII	carnitine palmitoyltransferase II
CS	citrate synthase
CSA	cross-sectional area
CyA	cyclosporine A
Cyt	cytochrome
DLCO or **TLCO**	diffusing capacity of carbon monoxide
DLTx	double lung transplantation
EDTA	methylenediaminetetra-acetic acid
ETC	electron transfer chain
ETFP	electron-transferring flavoprotein
FAD	flavin adenine dinucleotide
$FADH_2$	reduced form of flavin adenine dinucleotide
Fe-S	iron-sulfur centre
FEV_1	forced expiratory volume in first second
FFA	free fatty acid
FI	fatigue index
FMN	flavin mononucleotide
$FMNH_2$	reduced form of flavin mononucleotide
FVC	forced vital capacity
GDH	glutamate dehydrogenase
GDHim	the activity of GDH in intact mitochondria
GDHma	activity of GDH in mitochondrial suspension after rupture membrane
GDHmb	activity of GDH in mitochondrial suspension before rupture membrane
GDHt	total GDH activity

GPX	glutathione peroxidase
H_2O_2	hydrogen peroxide
HAD	3-hydroxyacyl-CoA dehydrogenase or β-hydroxyacyl-CoA dehydrogenase
Hb	haemoglobin
HCL	hydrochloric acid
HK	hexokinase
HLA	human lymphocyte antigen
HLTx	heart lung transplantation
HMGRIs	the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor
$HO_2^{\bullet}$	hydroperoxyl radical
HP	hypertension
HPLC	reverse phase high performance liquid chromatography
HRpeak	peak heart rate
IMP	inosine monophosphate
IPF	idiopathic pulmonary fibrosis
KGDH	2-oxoglutarate dehydrogenase
LDH	lactate dehydrogenase
LT	lactate threshold
LTx	lung transplantation
MAPR	mitochondrial ATP production rate
MDA	malondialdehyde
MDH	malate dehydrogenase
MOS	the Medical Outcomes of Health Survey
MPT	mitochondrial permeability transition
mtDNA	mitochondrial deoxyribonucleic acid
mtRNA	mitochondrial ribonucleic acid
MVV	predicted maximum voluntary ventilation
NAD	nicotinamide adenine dinucleotide
NADH	reduced form of nicotinamide adenine dinucleotide
NADPH	reduced form of nicotinamide adenine dinucleotide phosphate
NH_3	ammonia
Nm	Newton-meters
NMR	nuclear magnetic resonance
NO	nitric oxide
$O_2^{\bullet}$	superoxide anion
OB	obliterative bronchiolitis
$ONOO^{\bullet}$	Peroxynitrite
P + M	pyruvate + malate
P/O ratio	(number of molecules of Pi taken up to phosphorylate ADP, per atom of oxygen consumed)
P_{A-a} O_2 gradients	alveolar O_2 pressure gradients
PaO_2	arterial O_2 partial pressure
PC + M	palmitoyl-L-carnitine + malate
PCA	perchloric acid
Pco_2	carbon dioxide partial pressure
PCr	phosphocreatine
PDH	pyruvate dehydrogenase
PFK	phosphofructokinase
PHOSPH	phosphorylase
P_i	inorganic phosphate
PK	pyruvate kinase

PO_2	partial pressure of oxygen
PPH	primary pulmonary hypertension
PPKM	pyruvate + malate + palmitoyl-L-carnitine + α-ketoglutarate
R•	alkyl radical (R•; an abbreviation for organic molecules in general)
RISHLT	the Registry of the International Society for Heart and Lung Transplantation
ROO•	peroxyl radical
ROOH	hydroperoxide
ROS	reactive oxygen species
rpm	revolutions per minute
RT	resistance-trained subjects
S + R	succinate + rotenone
SaO_2	oxygen saturation
SDH	succinate dehydrogenase
SLTx	single lung transplantation
SEM	standard error of the mean
SOD	superoxide dismutase
SR	sarcoplasmic reticulum
TCA	tricarboxylic acid
TAN	total adenine nucleotides (ATP + ADP + AMP)
TCR	total creatine (PCr + Cr)
TG	triglycerides
TVR	the torque-velocity relationship
UQ	ubiquinone (coenzyme Q)
UQH_2	ubiquinol
UT	untrained controls.
UWW	underwater weigh % body fat
$\dot{V}C$	vital capacity
$\dot{V}CO_2$ peak	peak CO_2 uptake
$\dot{V}E$	minute ventilation
$\dot{V}E$ peak	minute ventilation at peak exercise
$\dot{V}O_2$ **max**	maximal O_2 uptake
$\dot{V}O_2$ **peak**	peak O_2 uptake

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIXES

CHAPTER 1. INTRODUCTION

It is now widely recognised that mitochondria are organelles vital for life and have a central function in energy supply in cells. Energy is the life source of living organisms and cells and activates growth, reproduction and activity. Mitochondria use fuels, such as carbohydrate, fatty acids and amino acids, to provide energy for cellular processes. Usually, these fuels are used to synthesise molecules of adenosine triphosphate (ATP) which are the chemical form of energy used in cells. ATP is referred to as a "high-energy phosphate compound" a term derived from the fact that hydrolysis of ATP to adenosine diphosphate (ADP) results in a large free energy change. Free energy resulting from this reaction is used to drive numerous processes, such as cell motility, muscle contraction and the specific transport of substances across membranes. The process of ATP production in mitochondria is termed oxidative phosphorylation and it involves transferring electrons along a chain of carriers, the so-called the electron transfer chain (ETC). The components of the ETC transfer electrons to oxygen from compounds, such as the reduced form of the coenzymes nicotinamide adenine dinucleotide ($NADH + H^+$) and flavin adenine dinucleotide ($FADH_2$). Simultaneously, protons are actively transported across the inner mitochondrial membranes creating a proton gradient. The processes of electron transport and proton pumping ultimately lead to the synthesis of ATP and H_2O.

Mitochondrial studies have been widespread in biology, physiology, pharmacology, and clinical medicine. It has been demonstrated that alterations in mitochondrial structure, function and mutation of mitochondrial deoxyribonucleic acid (mtDNA) influence the normal functions of cells (Holt et al 1989). Many disease states are also associated with, or due to, alteration of mitochondrial function (Luft 1995).

Impaired oxidative capacity has long been held to be a main limiting factor in patients with severe respiratory disease, and usually attributed to impaired pulmonary O_2 exchange with a

consequence depressed muscle O_2 delivery. Over the past two decades, heart-lung transplantation (HLTx) and lung transplantation (LTx) have been successful in treating end-stage pulmonary diseases. These treatments have not only improved the survival rate of recipients but also their quality of life (Williams and Snell 1997). The recipients of HLTx and LTx, however, have a poor exercise capacity. These patients have low endurance exercise tolerance, a low maximal oxygen consumption ($\dot{V}O_2$max) or peak oxygen uptake ($\dot{V}O_2$peak) and an early increase in lactate concentration in blood during incremental exercise tests (Miyoshi et al 1990; Williams et al 1992a; 1992b; Levy et al 1993; Orens et al 1995). Post transplantation pulmonary spirometry measurements indicate recovery of lung function to near normal levels (Madden et al 1992; Bando et al 1995), but exercise capacity in these patients remains lower than predicted levels for their age and sex. Therefore, it has been suggested that skeletal muscle dysfunction may be a major contributing factor to the exercise limitation in LTx patients (Williams and Snell 1997). Mitochondria may be impaired in skeletal muscle in these patients, and this may lead to alteration of the muscle metabolism during exercise (Howard et al 1994; Williams and Snell 1997). This decrease in mitochondrial capacity may arise as a result of numerous factors, including muscle deconditioning cause by prolonged inactivity, hypoxaemia and anaemia, imunosuppressive drugs. However, few studies have examined mitochondrial function of skeletal muscle in LTx patients.

Cyclosporine A (CyA) is an effective immunosuppressive agent, which is widely used in anti-rejection treatment for transplantation patients (Oyer et al 1983; Andreone et al 1986; Fragomeni and Kaye 1988). CyA, however, inhibits mitochondrial oxygen consumption of renal cell of rats (Jung and Pergande 1985) and humans (Jung and Reinholdt 1987). CyA at relatively high concentration also inhibits mitochondrial O_2 consumption of skeletal muscle in rats (Hokanson et al 1995). It has been demonstrated, in rats, that CyA induced skeletal muscle mitochondrial dysfunction is correlated with the limitation of exercise endurance performance (Mercier et al 1995). There are no studies, however, have examined the effect of

CyA at concentration likely be found in the muscle tissue of transplant recipients. In addition, there are no studies that have been examined the effects of CyA on mitochondrial ATP production rate (MAPR) in skeletal muscle.

Resistance training is an effective exercise mode to enhance muscle mass and muscle strength in healthy subjects (MacDougall et al 1980). Previous studies have demonstrated that a period of resistance training results in skeletal muscle fibre hypertrophy, associated with an improved muscle function, such as peak torque (MacDougall et al 1982; Houston et al 1983; Narici et al 1996). There are reports that resistance training can also improve endurance performance (Hickson et al 1980, 1988; Rube & Secher 1990). Increased $\dot{V}O_2$ peak and capillary supply in skeletal muscle has also been reported after resistance training in young (23-35 years-old; Gettman et al 1979; Hickson et al 1980) and older (65-75 years-old; Hepple et al 1997) subjects. Resistance training is especially effective on those subjects who have experienced bed rest (Ferrando et al 1997) and muscle disuse due to immobilisation (MacDougall et al 1980; Ingemann-Hansen and Halkjaer-Kristensen 1985a; 1985b; 1985c). It has also been widely used in rehabilitation programs for aged subjects (Sipilä and Suominen 1995; Tracy et al 1999). Recently, resistance training has been demonstrated as a safe and effective exercise mode to improve the exercise capacity in patients with cardiovascular disease (Magnusson et al 1996; McCartney and McKelvie 1996; Maiorana et al 1997; McCartney 1998). As a result, resistance training has also become particularly popular in rehabilitation programs for patients with cardiopulmonary diseases (Verrill and Ribisl 1996). Resistance training may be also a safe and effective exercise mode for lung transplantation patients.

Previous studies have shown that resistance training decreased (MacDougall et al 1979; 1982), increased (Staron et al 1984) or had no effect (Lüthi et al 1986) on mitochondrial volume density. Similarly, oxidative enzyme activity in skeletal muscle was decreased (Tesch et al 1987), increased (Costill et al 1979) and not changed (Schantz and Källman 1989; Green et al 1999) after resistance training. There have been no studies examining the effects of

resistance training on MAPR in human skeletal muscle. Therefore, it is necessary to determine the effects of resistance training on oxidative capacity or mitochondrial capacity in skeletal muscle.

The general aim of this thesis is to help in the understanding of the mechanisms underlying exercise limitation after lung transplantation and to evaluate the effects of resistance training on skeletal muscle mitochondria, which may assist in the design of rehabilitation programs following long-term immobilisation or heart and lung diseases. Accordingly, this thesis will examine:

(1). Muscle fibre type proportions, resting muscle metabolism, muscle mitochondrial enzyme activities and MAPR in a group of LTx patients (Chapter 4).

(2). The effects of CyA *in vitro* on MAPR in rat skeletal muscle (Chapter 5).

(3). Metabolism and performance during intense one leg exercise and incremental cycling exercise, muscle fibre type proportions, resting and fatigued muscle metabolism, muscle mitochondrial enzyme activities and MAPR in a group of resistance-trained subjects (Chapter 6).

CHAPTER 2. LITERATURE REVIEW

Skeletal muscle contractions depend upon energy which is supplied from two broad processes of energy metabolism in skeletal muscle. Those processes requiring oxygen are termed aerobic metabolism and those not requiring oxygen are termed anaerobic metabolism. Each of these processes plays an important role during exercise. Mitochondria are the locations of aerobic metabolism in cells. In skeletal muscle, mitochondrial capacity can be affected by a numbers of factors, such as the age, muscle fibre type, and physical activity. Patients with long-term heart and lung diseases have a low level of exercise capacity, associated with a low type I fibre proportion, low mitochondrial enzyme activity and metabolites abnormalities in skeletal muscle. Although heart and lung transplantation can restore the cardiopulmonary function in these patients, the endurance exercise tolerance and maximal oxygen uptake in exercise are still lower than predicted levels. Potential reasons for exercise limitation in these transplant patients include central organ (heart and lung) function incompetence, peripheral skeletal muscle deconditioning and toxicity caused by the immunosuppressant drugs. Accordingly, the following review will discuss:

- Skeletal muscle metabolism in exercise.
- Mitochondrial structure and function.
- Factors affecting mitochondrial capacity.
- Post HLTx and LTx outcomes, exercise limitation and its potential causes.

2.1. SKELETAL MUSCLE METABOLISM IN EXERCISE

2.1.1. ATP Resynthesis in Exercise

ATP is a molecule with a structure allowing for capture, storage and transport of free energy

in all living cells. During exercise, ATP is the immediate donor of energy for skeletal muscle contraction (Cain et al 1962). The amount of ATP stored in muscle, however, is very limited [~24 mmol kg^{-1} dry wt. of ATP (Hultman 1967)], sufficient only for a few seconds of high intensity exercise. Therefore, ATP resynthesis is an important metabolic process in order to maintain cellular ATP and thereby provide the energy required for muscle contraction during exercise.

During exercise, skeletal muscle ATP resynthesis occurs via both aerobic and anaerobic metabolism. The proportional contribution to ATP production from aerobic and anaerobic processes is dependent upon the duration and intensity of exercise. For example, Bangsbo et al 1990 examined exercise metabolism during knee-extensor intense exercise to exhaustion (65W). They found that, in the first 30 seconds of exercise, ~80% of the total ATP is derived from anaerobic sources, but this figure declined to 30% after 3.2 minutes exercise. During the prolonged exercise the ATP production comes predominantly through aerobic metabolism (Sahlin et al 1990). Low intensity exercise over 120 minutes approximately 99% of ATP production is derived from aerobic sources (Åstrand and Rodahl 1977).

2.1.2. Anaerobic Metabolism

Anaerobic metabolism consists of three pathways, the creatine phosphokinase pathway (Hultman 1967), adenylate kinase or myokinase pathway (Sahlin and Broberg 1990), and glycogenolysis and glycolysis which involve the breakdown of glucose or glycogen (Katz and Sahlin 1990). Anaerobic metabolism supplies a limited amount of ATP. For instance, glycolysis produces a net 2 ATP from glucose and 3 ATP from a glucosyl unit derived from glycogen. This compares with 36 net ATP from glucose oxidation and 37 net ATP from glucosyl unit oxidation in most tissues while oxygen supply is adequate. In certain circumstances, however, anaerobic metabolism is the dominant energy producing pathway.

Examples of such circumstances include the commencement of exercise and short-term high intensity exercise (Hultman 1967; Saltin 1973; Bangsbo et al 1990). It is also known that there is a greater capacity of anaerobic metabolism in muscle type II fibres, especially in types IIb fibres (Saltin et al 1977). In addition, the contribution of anaerobic metabolism to ATP supply in skeletal muscle can increase under conditions of hypoxia (Sahlin and Katz 1989), muscle ischaemia (Welsh and Lindinger 1997) and during exercise in the heat (Febbraio et al 1994).

2.1.2.1. Phosphocreatine Kinase Reactions

When skeletal muscle contraction begins, the muscle fibre obtains its immediate energy by splitting ATP to form adenosine diphosphate (ADP), protons (H^+) and inorganic phosphate (P_i). The process, termed ATP hydrolysis, is described in equation (1).

$$ATP \xrightarrow{ATPase} ADP + P_i + H^+ \quad (1).$$

Three major ATPases in skeletal muscle are Na^--K^+-ATPase, actinomyosin -ATPase and Ca^{2+}-ATPase which hydrolyse ATP to supply energy for muscle membrane excitation (Nielsen and Clausen 1997), force development (Matsunaga et al 1999) and relaxation (Hasselbach 1964), respectively. Following ATP hydrolysis, skeletal muscles can resynthesise ATP from phosphocreatine (PCr) stored in muscle (Hirvonen et al 1987), through a reaction catalysed by creatine phosphokinase (CPK) (Altschuld and Brierley 1977). This reaction is described in equation (2).

$$ADP + PCr + H^+ \xrightarrow{CPK} ATP + Cr \text{ (creatine)} \quad (2).$$

This process occurs immediately, at the onset of contraction (Gaitanos et al 1993). Muscle biopsy studies have demonstrated that muscle PCr content is largely degraded after 6 seconds intense cycling exercise before ATP content is depleted (Boobis et al 1982). Hence, phosphocreatine buffers ATP concentrations early in exercise (Sahlin et al 1998), but only a limited amount of PCr (67.8 mmol kg^{-1} dry wt) is stored in skeletal muscle (Hultman 1967).

2.1.2.2. Adenylate Kinase or Myokinase Reactions

When the rate of ATP hydrolysis exceeds the rate of ATP synthesis, ADP concentration in the muscle cell will rise. This increased ADP will lead to the activation of the adenylate kinase (AK) or myokinase reaction (Sahlin and Broberg 1990). In this process, two molecules of ADP are converted to one of ATP and one of adenosine monophosphate (AMP) (Aragon and Lowenstein 1980). This process is described in equation (3).

$$ADP + ADP \xleftrightarrow{AK} ATP + AMP \qquad (3).$$

The increase in concentration of AMP and the decrease in the ratio of ATP/ADP activate the activity of AMP deaminase (Newsholme and Start 1973). AMP deaminase can convert AMP to inosine monophosphate (IMP) and ammonia (NH_3) (Jansson et al 1987; Sahlin et al 1989). This reaction is summarised in equation (4).

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

IMP accumulation is an indicator of disturbed balance of energy metabolism (Norman 1994). IMP is further convered to inosine and hypoxanthine which can be released from skeletal muscle into blood. (Hellsten-Westing et al 1994). During intense exercise, IMP accumulation

in muscle may reach 7.4 mmol min^{-1} kg^{-1} w.w. or about 90-fold increase resting level (Stathis et al 1994).

2.1.2.3. Anaerobic Glycogenolysis and Glycolysis Reactions

Glycogenolysis is the process of the breakdown of glycogen to glucose-1-phosphate. In this process, glucosyl units from glycogen are converted to glucose-1-phosphate by glycogen phosphorylase (PHOSPH). Glucose-1-phosphate is further converted to glucose-6-phosphate. The latter compound is ultimately metabolised to pyruvate in a series of reactions referred to as glycolysis. Glucose-6-phosphate can also be produced by phosphorylation of glucose, a reaction which is catalysed by the enzyme hexokinase (HK). These processes occur in the cytoplasm. The main reactions and enzymes in glycogenolysis or glycolysis are shown in Figure 2.1. The rate limiting step in glycolysis is thought to be the conversion of frucose-6-phosphate to frucose-1,6-bisphosphate via the enzyme phosphofructokinase (PFK) (Campbell et al 1987). The enzymes PHOSPH, HK, PFK, along with pyruvate kinase (PK) which catalyses the conversion of phosphoenolpyruvate to pyruvate, are the so-called "non-equilibrium" enzymes of the glycogenolytic and glycolytic reactions (Newsholme 1980). These enzymes provide direction in these pathways, are allosterically regulated and are considered quantitative indices of the maximum capacities of glycogenolytic and glycolytic metabolism.

In anaerobic metabolism, pyruvate is converted to lactate by lactate dehydrogenase. Lactate accumulates in skeletal muscle and is also released into the interstitium and blood stream during exercise (Karlsson and Saltin 1970). Elevated lactate production reflects an increased glycolytic rate (Wasserman et al 1973; Dodd et al 1993). An increase in lactate production, however, is also caused by other factors, such as an elevated adrenaline concentration in blood, which stimulates glycolytic reactions (Ren and Hultman 1989; Greenhaff et al 1991). Muscle fibre type composition influences lactate production. Lactate dehydrogenase (LDH)

activity (Essén and Henrikson 1980) and lactate production during exercise (Ball-Burnett et al 1991) are much higher in type II fibres compared with type I fibres.

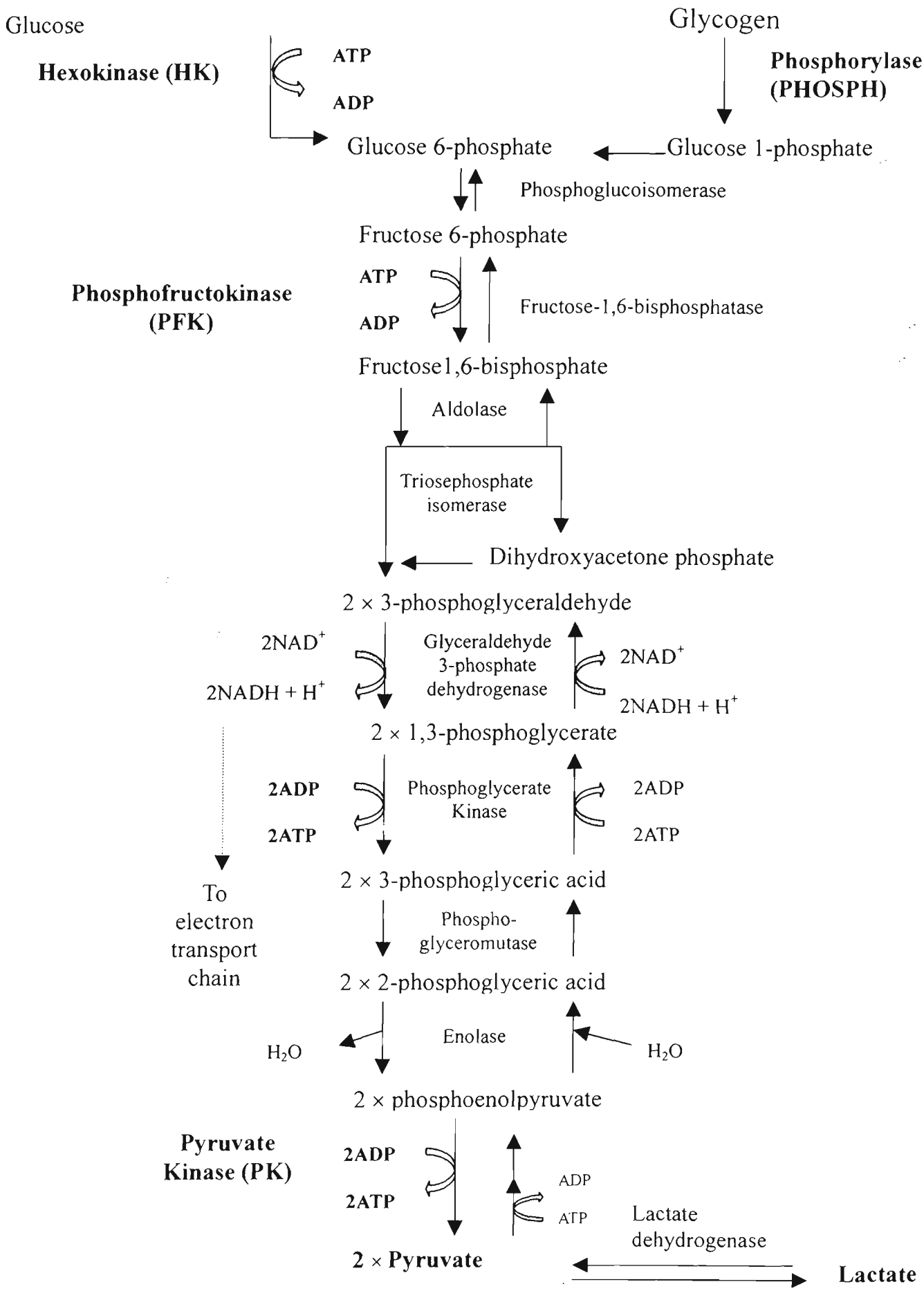


Figure 2.1. Pathway of Anaerobic Glycogenolysis and Glycolysis

2.1.3. Aerobic Metabolism

Aerobic metabolism occurs in mitochondria, where carbohydrate, fatty acid and amino acids are used as fuels to produce ATP. Oxidation of lipids in mitochondria is the main energy source at rest and during prolonged exercise (Costill et al 1977; Hurley et al 1986; Sahlin et al 1990). Carbohydrate oxidation is also important during prolonged exercise (Spencer et al 1991; Wagenmakers et al 1991). It has been demonstrated that carbohydrate depletion may impair aerobic energy production via reducing tricarboxylic acid (TCA) cycle intermediates during prolonged exercise (Sahlin et al 1990). Oxidation of amino acids in skeletal muscle makes a lesser contribution to energy supply during exercise (Hood and Terjung 1990). Amino acids, however, become important energy substrates when muscle glycogen (Blomstrand and Saltin 1999) and blood glucose concentrations (MacLean et al 1991) are low during prolonged exercise. The reactions of aerobic metabolism will be discussed in the section of mitochondrial function subsequently (see Section 2.2.2).

2.1.4. Summary

During exercise, ATP is the immediate source of energy for skeletal muscle contraction, but ATP storage in muscle is very limited. Therefore, ATP resynthesis is crucial metabolic process. Generally, resynthesis of ATP involves both anaerobic metabolism and aerobic metabolism. There are three anaerobic pathways responsible for ATP resynthesis: the phosphocreatine pathway, the adenylate kinase (AK) or myokinase pathway and glycolysis. ATP resynthesis via anaerobic metabolism is a very important source of energy during the initial part of exercise and for short and high intensity exercise. Aerobic ATP synthesis occurs in the mitochondria. Mitochondria produce most of the cells ATP requirements via aerobic metabolism. During exercise lasting for more than one minute or longer duration, most of

ATP resynthesis occurs in the mitochondria. Therefore, mitochondria are essential organelles to produce energy requirement for exercise.

2.2. Mitochondrial Structure and Function

Mitochondria are most commonly observed (Palade 1953) as oval-shaped organelles, typically about 1-2 μm in length and 0.5-1.0 μm in diameter (Figure 2.2, the mitochondrial membranes and structures will be discussed in the next Section). In skeletal muscle, mitochondria are arranged in rows located predominantly either just below the sarcolemma or between the myofibrils. Hence, some investigators have divided skeletal muscle mitochondria into two populations: subsarcolemmal and intermyofibrillar mitochondria (Hoppeler et al 1973; Krieger et al 1980). This traditional concept, however, has been challenged by another model. Bakeeva (1978) and Kirkwood et al (1986) suggested that mitochondrion is like an elaborate network or reticulum in skeletal muscle (Figure 2.3), rather than individual, capsule-shaped organelles. Each mitochondrion contains its own DNA and ribosomes, but most proteins are imported and target suitable positions (Hartl et al 1988). Division of mitochondria is the process for reproduction of new mitochondria (Fawcett 1994).

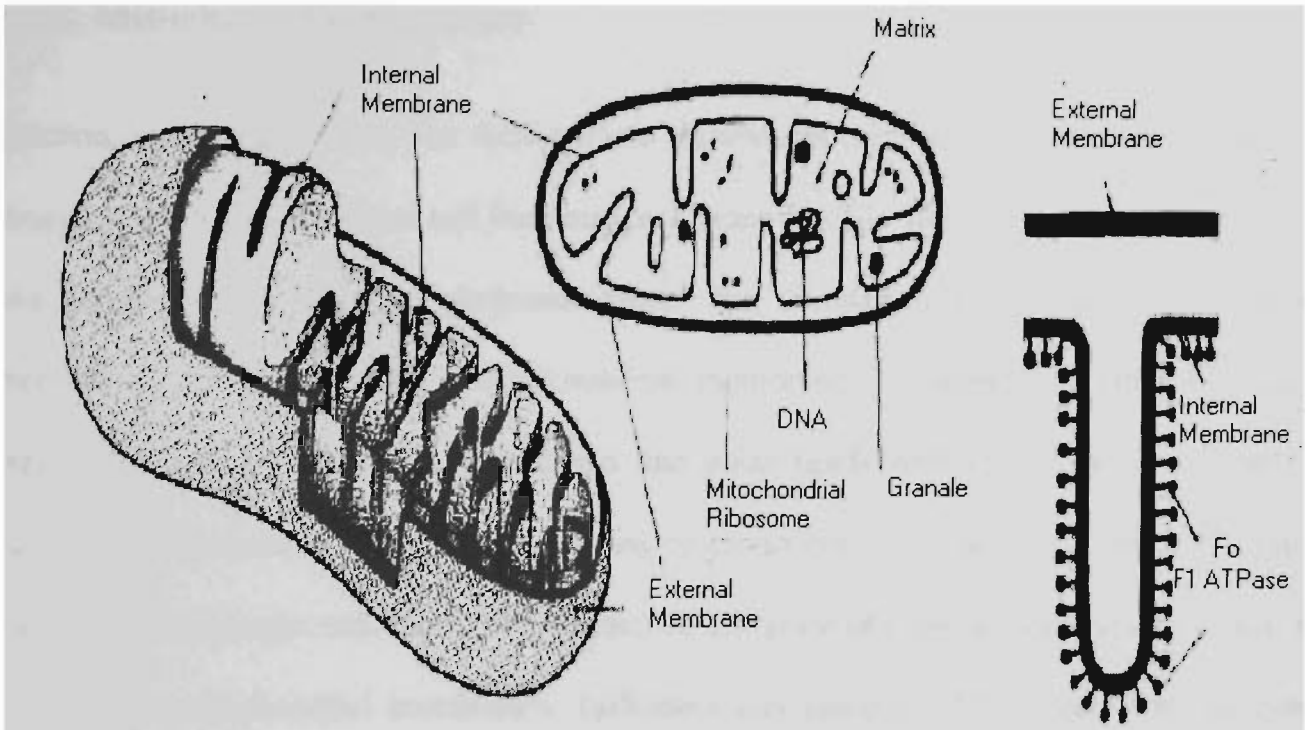


Figure 2.2. Mitochondrial Structure

Diagram is from Childs (1998)

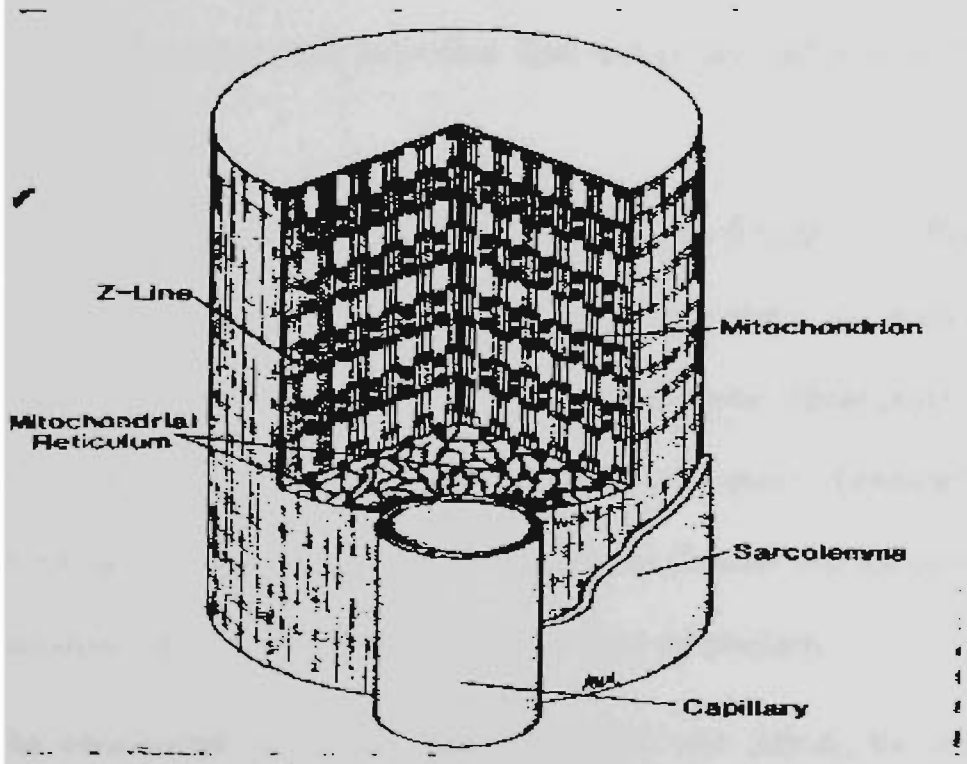


Figure 2.3. Mitochondrial Network

Diagram is from Kirkwood et al (1986)

2.2.1. Mitochondrial Structure

Electron microscopy reveals that there are two membranes enclosing the entire mitochondrial structure, the outer membrane and the inner membrane (Palade 1953). The space between the two membranes is the intermembrane space. The compartment enclosed by the inner membrane is called the matrix. Mitochondrial membranes are similar to other biological membranes and are composed of proteins and polar lipids with only a small amount of carbohydrate present as part of glycoproteins or glycolipids (Parsons et al 1967). The fluid mosaic model (Singer and Nicolson 1972) for the structure of plasma membrane may also be suitable for mitochondrial membranes. Gellerfors and Linden (1981) found that the outer mitochondrial membranes fragments to vesicles which have similar density and morphology to plasma membranes. In the fluid mosaic model of membrane structure, globular proteins of various sizes and with different orientations are embedded in the lipid bilayer (Singer and Nicolson 1972). Proteins which penetrate through the membrane are referred to as integral proteins. Other proteins, which are located on the surface of the membrane, are called peripheral proteins. The individual lipid and protein subunits in the membrane are free to move laterally.

The outer mitochondrial membrane is ~6-7 nm thick with ~50 different types of polypeptide components (Tyler 1995). Many of these polypeptides are parts of proteins which form special 2-4 nm diameter pores through the membrane. These pores allow most molecules of molecular weight less than 5,000 Dalton to pass easily (Lehninger et al 1993). The outer membrane also contains a number of enzymes (Ernster and Kuylenstierna 1970; Tyler 1995) including redox enzymes and enzymes of lipid metabolism.

The inner membrane is also about 6nm thick with infolds, the cristae, giving a very large surface area. The inner membrane has at least 100 distinct types of polypeptides and a high content of protein (Tyler 1995). Generally, this 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 1991). The inner membrane contains the components of the ETC which comprises complexes, I, II, III, IV and ATP synthase (Hatefi 1985). The membrane also contains a series of carriers for the transport of ions, substrates, and nucleotides (Sherratt 1991). Most of the carriers are integral membrane proteins which are buried within the membrane but are exposed on both faces of membrane. Examples of carriers include ADP-ATP translocase (Tyler 1995). The inner membrane of a single liver mitochondrion may have over 10,000 sets of the ETC. A heart mitochondrion contains over three times the number of sets of respiratory chains compared with a liver mitochondrion, due to the profusion of cristae which creates a much larger area of the inner membrane (Lehninger et al 1993).

The intermembrane space contains some enzymes such as adenylate kinase (AK), nucleoside diphosphate kinase and nucleoside monophosphokinase (Ernster and Kuylenstierna 1970).

The matrix is the inner compartment of the mitochondrion. It contains many enzymes and chemical intermediaries involved in energy-yielding metabolism. There are at least 200 different types of polypeptides in the matrix space (Tyler 1995). Enzymes of significance are as follows: pyruvate dehydrogenase, the TCA enzymes, fatty acid oxidation system enzymes, amino acid oxidation enzymes, various aminoacyl transferases, DNA polymerase and ribonucleic acid (RNA) polymerase, protein synthesis, some heme synthesis, superoxide dismutase. The function of these enzymes or enzyme systems relevant to this thesis will be discussed subsequently.

2.2.2. Mitochondrial Function

2.2.2.1. Overview of Oxidation in Mitochondria

The oxidative processes in mitochondria comprise three major stages (Figure 2.4) as below:

- Oxidation of fatty acids, some amino acids and pyruvate derived from carbohydrate, to form the acetyl group of acetyl-CoA.
- Enzymatic oxidation of acetyl-CoA to CO_2 with the formation of reduced electron carriers NADH and $FADH_2$ via the TCA cycle.
- NADH + H^+ and $FADH_2$ carry electrons into the mitochondrial ETC, ultimately forming H_2O as well as producing ATP by chemiosmotic oxidative phosphorylation.

2.2.2.2. Oxidation of Fuels

Carbohydrate Oxidation

Cells degrade carbohydrates to form pyruvate in the cytosol without requiring O_2. The pyruvate is carried by the monocarboxylate carrier into the mitochondrial matrix where it is pyruvate oxidation to acetyl-coenzyme A (Acetyl-CoA), NADH + H^+ and CO_2. The monocarboxylate carrier is a specific carrier protein located in the inner membrane of mitochondria (Tyler 1995). The reaction is catalysed by the pyruvate dehydrogenase (PDH) complex (Denton and Halestrap 1979) as indicated in equation (5):

$$\text{Pyruvate} + \text{CoA-SH} + NAD^+ \xrightarrow{\text{PDH}} \text{Acetyl-CoA} + NADH + H^+ + CO_2 \qquad (5).$$

Acetyl-CoA is the form in which the TCA cycle accepts its fuel input (McGarry and Foster 1980).

Fatty Acid Oxidation

Fatty acid oxidation consists of two main processes, fatty-acid activation and β-oxidation (Schulz 1991). In the activation process fatty acids react with ATP and CoA to form acyl-CoA and AMP in equation (6):

$$\text{Fatty acids} + \text{ATP} + \text{CoA} \longleftrightarrow \text{Acyl-CoA} + \text{AMP} + \text{PP}_i \qquad (6).$$

Long chain fatty acids (C_{16} to C_{20}) are activated by long-chain fatty acyl-CoA synthetase which is located in the outer membrane (Tanaka et al 1979). The transport of activated long-chain fatty acid across the mitochondrial inner membrane requires carnitine palmitoyltransferase (CPTI) situated in the outer membrane of mitochondria and a second carnitine palmitoyltransferase (CPTII) located in the inner membrane of mitochondria (McGarry and Foster 1980). Because the mitochondrial inner membrane is impermeable to long-chain acyl-CoA molecules. Medium (C_4 to C_{11}) and short-chain-length fatty acids, however, can cross the inner membrane and are activated by short-chain and medium-chain acyl-CoA synthetase which are located in the mitochondrial matrix (Groot et al 1976).

The process of fatty acid oxidation is termed *β*-oxidation since it occurs through the sequential removal of 2-carbon units by oxidation at the β-carbon position of the fatty acyl-CoA molecule. *β*-oxidation comprises four reaction steps (Schulz 1991, see Figure 2.5). The enzyme *β*-hydroxyacyl CoA dehydrogenase or L-3-hydroxyacyl CoA dehydrogenase (HAD) is involved *β*-oxidation and catalyzes L-3-hydroxyacyl-CoA to 3-oxoacyl-CoA (Osumi and Hashimoto 1980, see Figure 2.5). The *β*-oxidation of one molecular of long chain fatty acids can produce more acetyl-CoA, NADH + H^+ and $FADH_2$ than oxidation of glucose (see Section 2.2.2.4). For example, the complete oxidation of one palmitate molecule (C_{16}) can produce 8 acetyl-CoA, 7 NADH + H^+ and 7 $FADH_2$ and gives total net 129 molecules of ATP. Obviously, fatty acid oxidation can supply a large amount of ATP. The process of the long-

chain saturated fatty acid activation and β-oxidation is shown in Figure 2.5.

Amino Acid Oxidation

As showing in Figure 2.6a, amino acids are also converted to acetyl-CoA, succinyl-CoA, oxaloacetate, fumarate and α-ketoglutarate, which then become substrates of the TCA cycle reactions (Felig 1975). In the metabolism of amino acids, the coupled action of the aminotransferase and glutamate dehydrogenase (GDH) (called transamination), provides one of major routes for the conversion of amino acids into oxyacids and ammonia (Goodman and Lowenstein 1977). Most of the aminotransferase reactions occur in the cytosol, but GDH is located in the mitochondrial matrix (Figure 2.6b). For example, the amino acid glutamine can be converted by aminotransferase to glutamate in the cytosol. The glutamate is transported by glutamate carrier (Glu carrier), which located in the inner membrane, into the mitochondrial matrix and is converted by GDH into α-ketoglutarate, ammonia and NADH + H^+ (Tyler 1995). The α-ketoglutarate, produced in this reaction, can be transported by α-ketoglutarate carrier to the cytosol to continue this process (Figure 2.6b) or used as an intermediate in the TCA cycle (Figure 2.6a).

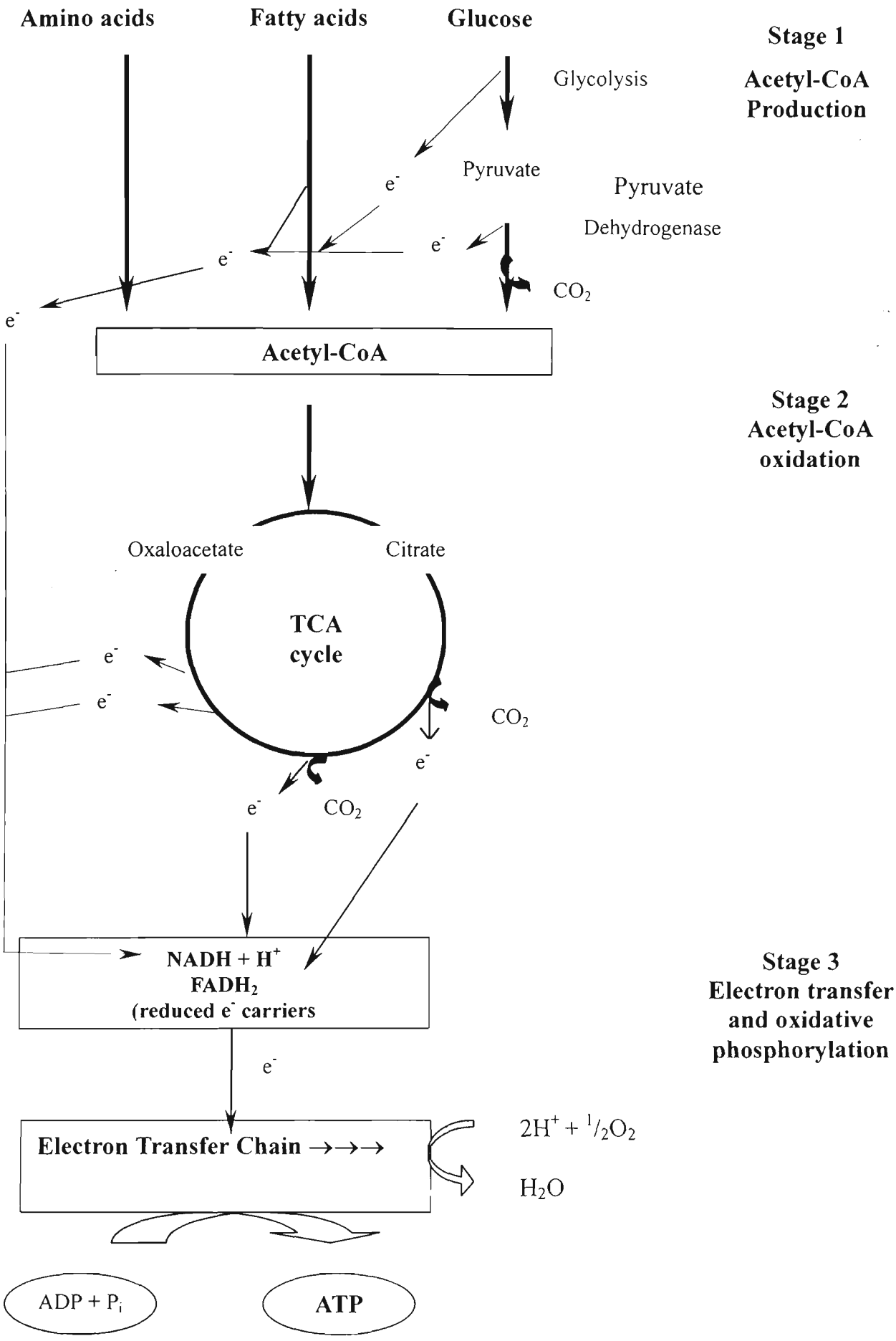


Figure 2.4. Catabolism of Proteins, Fats, and Carbohydrates in Cellular Respiration

Diagram is redrawn from Lehninger et al (1993), p 447. TCA, tricarboxylic acid cycle.

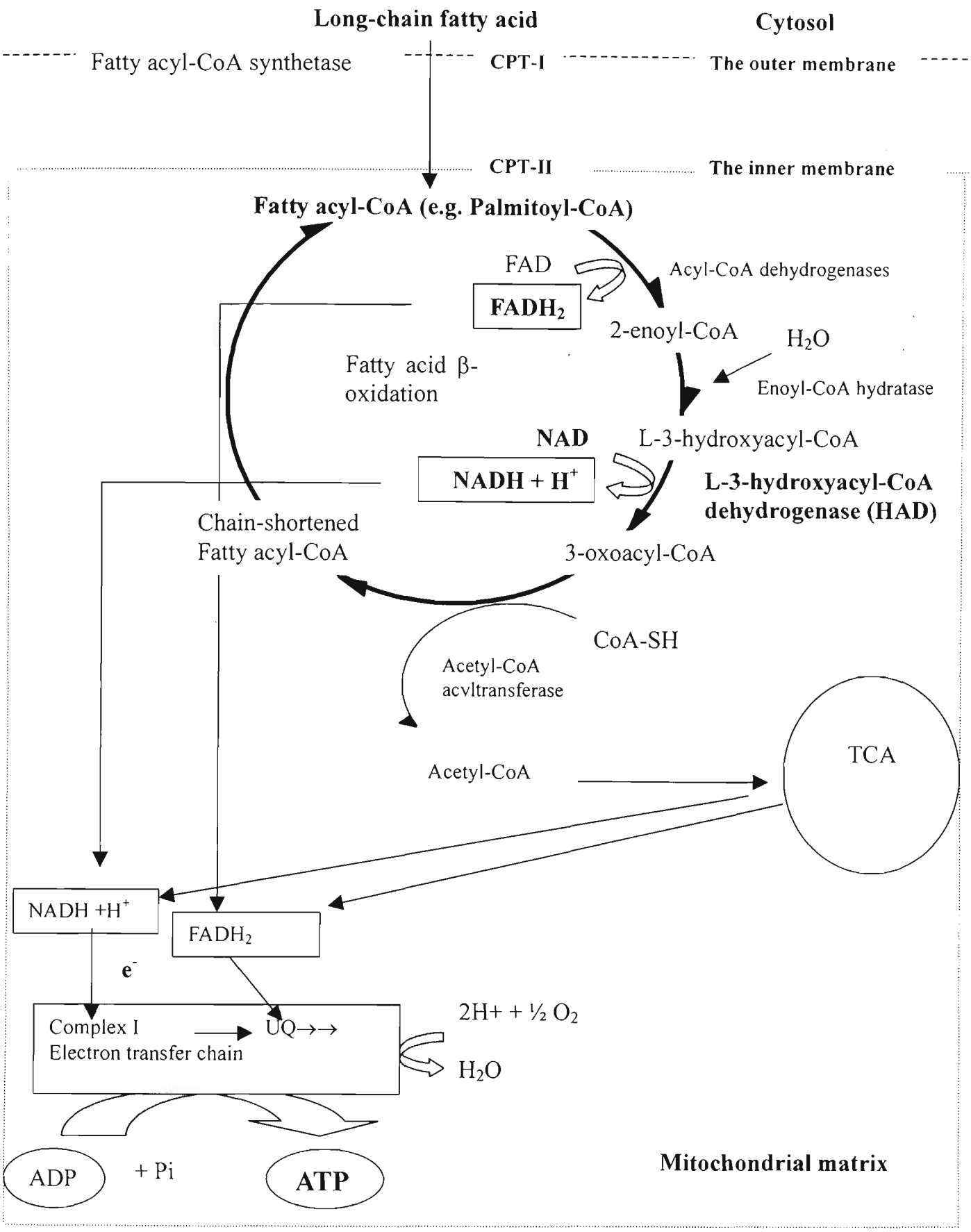
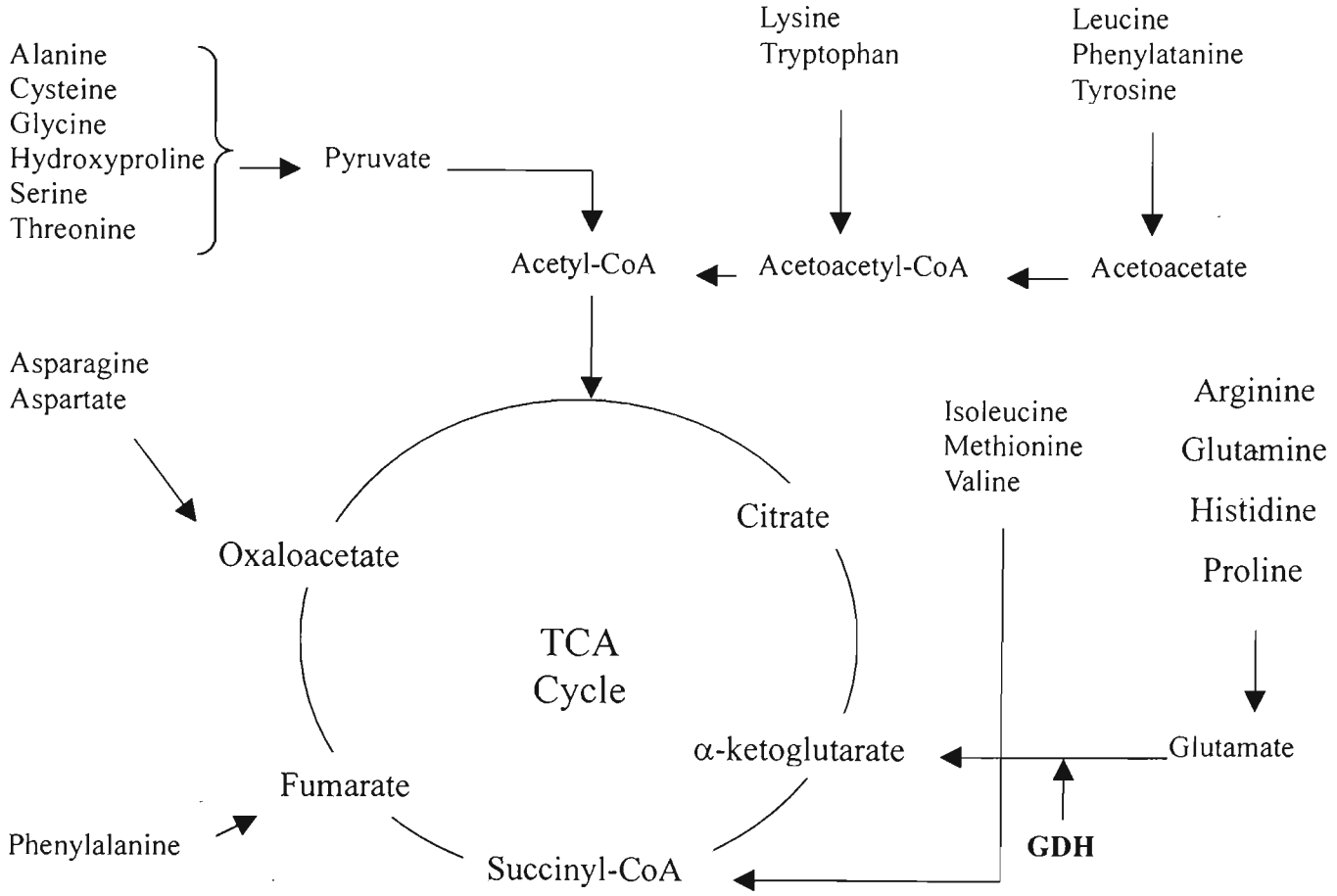
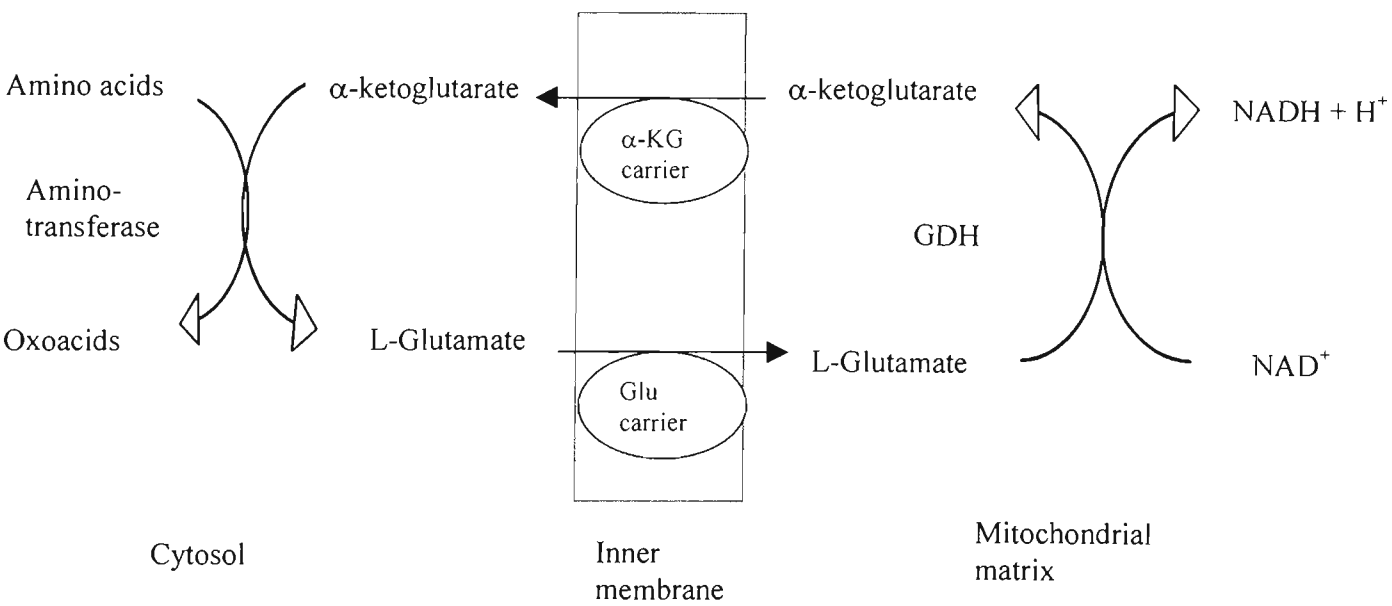


Figure 2.5. Fatty Acid Activation and β-oxidation

CPT, carnitine palmitoyltransferase; TCA, Tricarboxylic acid cycle.



(a) Production of acetyl-CoA and intermediate in the TCA cycle from amino acids.



(b) Aminotransferase and GDH reactions

Figure 2.6. Oxidative Reactions of Amino Acids

Diagrams redrawn from Tyler (1995), Pp 84 & 82. GDH, Glutamate dehydrogenase. TCA, Tricarboxylic acid cycle.

2.2.2.3. The Tricarboxylic Acid Cycle

Enzymes in the tricarboxylic acid cycle (TCA cycle), also termed citric acid cycle or Krebs cycle, are located in the mitochondria (Krebs and Johnson 1937; Green et al 1948). There are eight reaction steps in the TCA and each of the steps is catalyzed by a specific enzyme or enzyme complex as shown in Figure 2.7. The enzyme citrate synthase (CS) catalyzes the first step of TCA in which oxaloacetate combines with acetyl-CoA to produce citrate and CoA-SH (Shepherd and Garland 1969). The enzyme α-ketoglutarate dehydrogenase (KGDH) catalyzes the fourth step of TCA cycle in which α-ketoglutarate combine with CoA-SH to produce succinyl-CoA, $NADH+H^+$ and CO_2 (Yeaman 1989). These are two key nonequilibrium enzymes in the TCA cycle (Newsholme 1983) and they can, therefore, provide information about the maximum capacity of the cycle. Although the TCA cycle itself directly generates only one molecule of ATP or GTP per turn, the four oxidation steps produce 3 molecules $NADH + H^+$ and 1 molecule $FADH_2$. The $NADH + H^+$ and $FADH_2$ act as electron carriers to the respiratory chain. Consequently, a large number of ATP molecules are ultimately produced as a result of the TCA cycle.

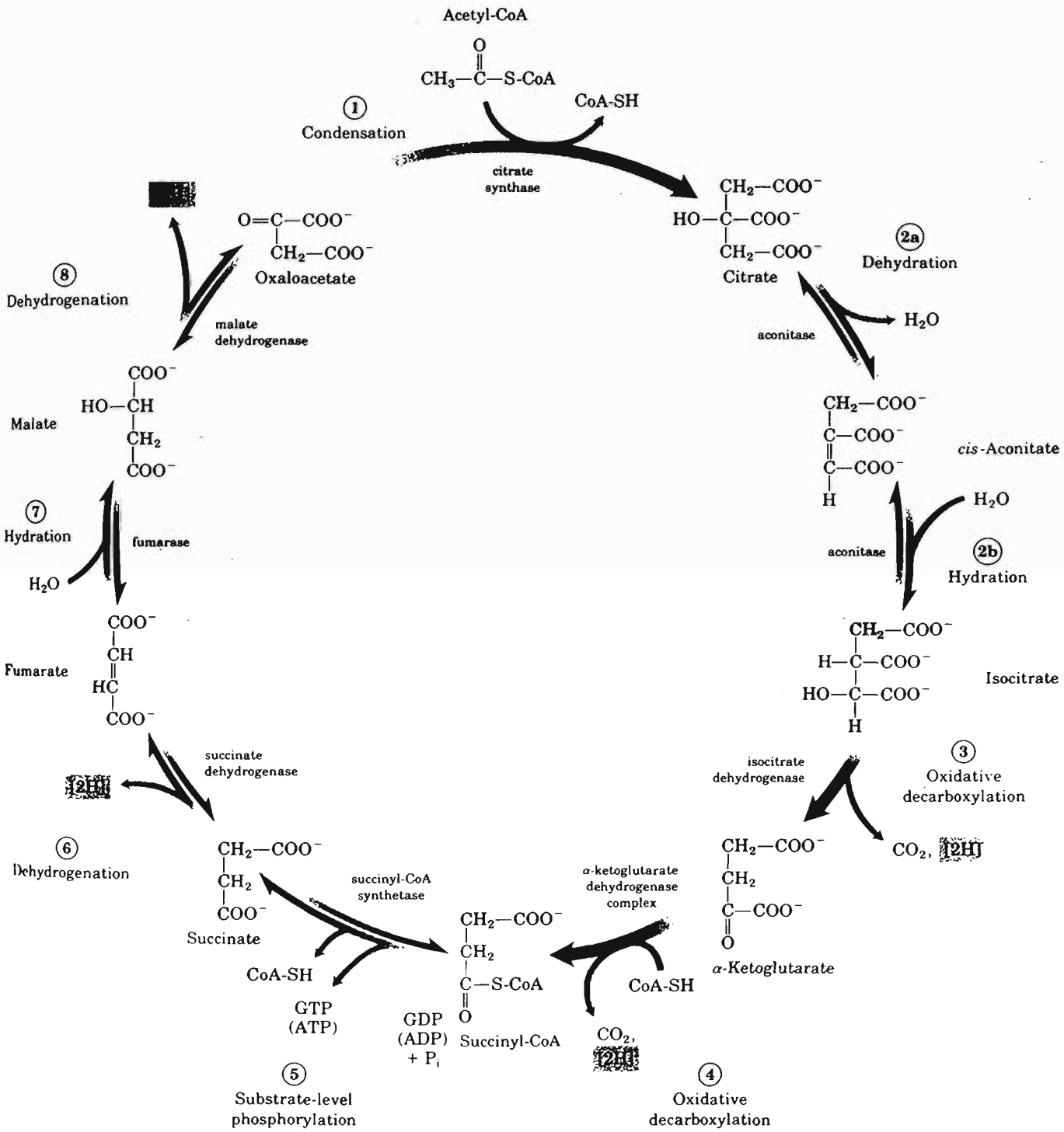


Figure 2.7. The Tricarboxylic Acid (TCA) Cycle

1. Formation of citrate. 2a and 2b. Formation of isocitrate via cis-aconitate. 3. Oxidation of isocitrate to α-ketoglutarate and CO_2. 4. Oxidative α-ketoglutarate to succinyl-CoA and CO_2. 5. Conversion of succinyl-CoA to succinate. 6. Oxidation of succinate to fumarate. 7. Hydration of fumarate to produce malate. 8. Oxidation of malate to oxaloacetate. Diagram is redrawn from Lehninger et al (1993).

2.2.2.4. Oxidative Phosphorylation and Mitochondrial Electron Transfer Chain

Transferring electrons to oxygen to drive ATP synthesis is termed oxidative phosphorylation (Chance and Williams 1955). This process involves the reduction of O_2 to H_2O with electrons supplied by NADH + H^+ and $FADH_2$. These electrons are transfer through a chain of complexes termed the mitochondrial respiratory chain (Chance and Williams 1956) or mitochondrial electron transfer chain (ETC) (Green and Tzagoloff 1966). This system contains a number of redox cofactors or electron carriers, such as, nicotinamide adenine dinucleotide (NAD), flavin, haem iron, sulfur iron, copper ions, cytochrome and ubiquinone (UQ) or coenzyme Q (Hatefi et al 1962a; 1962b; Hatefi 1985; Babcock and Wikström. 1992). These carriers which are shown in Figure 2.8, are bound into proteins in the ETC and form the four respiratory chain components, known as Complexes I, II, III, and IV (Hatefi 1985). There is another complex called ATP synthase for ATP formation (Racker et al 1965). Some researchers refer to ATP synthase as Complex V in the ETC (Capaldi 1982). The main characteristics of the ETC complexes are described below:

Complex I, or NADH dehydrogenase, or NADH: ubiquinone oxidoreductase, has a molecular weight of ~850 kDa in mammalian mitochondria (Capaldi 1982). This complex is a L-shaped single holoenzyme molecule (Hofhaus et al 1991) and receives electrons from NADH + H^+. Complex I transfers a pair of electrons through its flavin mononucleotide (FMN) and iron-sulfur centres (Fe-S) to UQ to form reduced ubiquinone (UQH_2) (Weiss et al 1991). The reaction sequence of Complex I is summarised in equation (7).

$$\begin{array}{cccccc} NADH+ & & FMN & & 2\ Reduced\ Fe\text{-}S & & UQ \\ & \curvearrowright\ \curvearrowleft & & \curvearrowleft\ \curvearrowright & & \curvearrowright\ \curvearrowleft & \\ NAD^+ & & FMNH_2 & & Oxidized\ Fe\text{-}S & & UQH_2 \end{array} \qquad (7).$$

This reaction of Complex I in ETC can be inhibited by rotenone which is called site I inhibitor (Vanden Hoek et al 1997). As previously indicated that the oxidation of carbohydrate, fatty acids and amino acids produces NADH + H^+ via the TCA cycle and β-oxidation in mitochondria. The NADH + H^+ enter the ETC through Complex I (Figure 2.8).

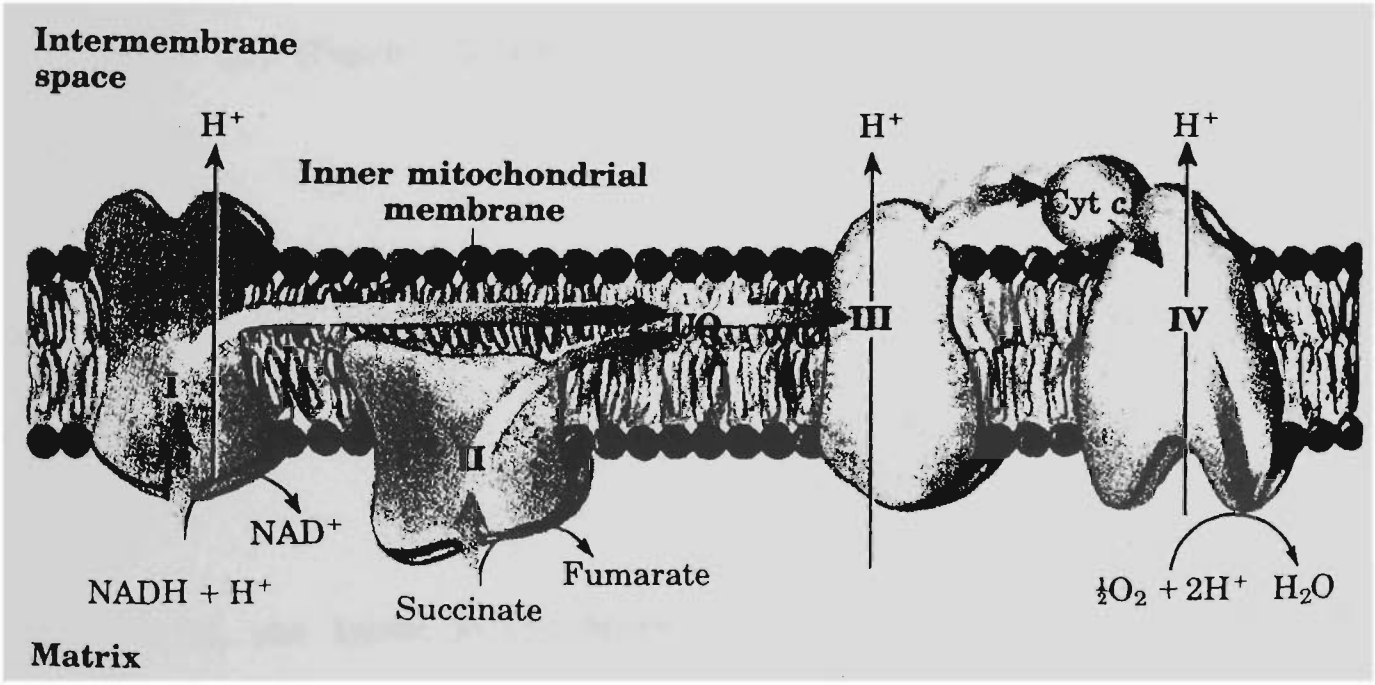
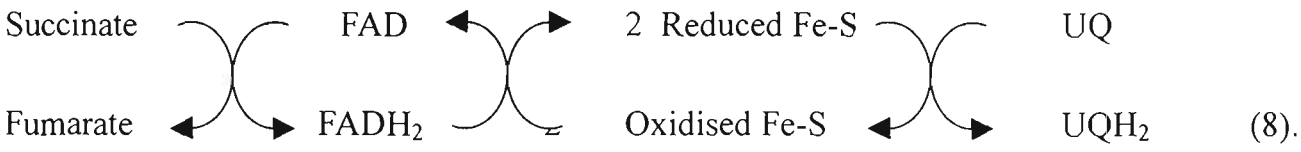


Figure 2.8. The Components of the Mitochondrial Electron Transfer Chain

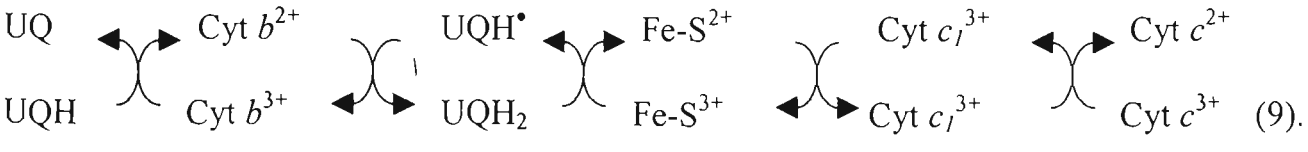
Diaphragm is from Lehninger et al (1993) Pp 554, I, Complex I (NADH-CoQ reductase); II, Complex II (Succinate-CoQ reductase); III, Complex III (ubiquinol-cytochrome-c reductase); IV, Cytochrome-c oxidase; UQ, ubiquinone (coenzyme Q).

Complex II is also named succinate-Q reductase complex or succinate: ubiquinone oxidoreductase. This complex has molecular weight ~120 kDa (Capaldi 1982) and is located on the matrix side of the inner membrane. Complex II contains one flavin adenine dinucleotide (FAD), three Fe-S centres and one cytochrome *b* (Hatefi 1985). It can be divided into two fractions: the hydrophobic peptide with cytochrome b_{560} and the enzyme succinate dehydrogenase (SDH) (Hatefi and Galante 1980). The latter is the major component of Complex II. As showed in Figure 2.8, Complex II catalyses electrons transfer from succinate to FAD and subsequently, the electrons are transferred to UQ (Hatefi 1985). Since succinate oxidation occurs via Complex II, it has been used as substrate to determine the function of this Complex *in vitro* (Figure 2.9). The reaction sequence of Complex II is summarised in equation (8).



(8).

Complex III, also known as cytochrome *c* reductase, cytochrome bc_1 complex and ubiquinone-cytochrome-c reductase, has a molecular weight of ~280 kDa and ~10-11 peptide subunits (Capaldi 1982). There are four redox centres including two haems b_H and b_L in cytochrome *b*, a 2Fe-2S center in the iron-sulfur protein centre, and a haem in cytochrome c_1. A pair of electrons is transferred from UQH_2 to two molecules of cytochrome *c* (Trumpower 1990). Antimycin A can block electron transfer from cytochrome *b* to cytochrome c_1 in this complex (Hatefi et al 1962a). Therefore, antimycin A is termed a site II inhibitor (Vanden Hoek et al 1997). The reaction sequence of Complex III is summarised in equation (9).



(9).

Cytochrome *c* is located in the intermembrane space and is a relatively small water-soluble protein (~13 kDa) comprising a single polypeptide chain (Capaldi 1982). The main function of cytochrome *c* is to transfer electrons from Complex III to Complex IV.

Complex IV, or cytochrome *c* oxidase has a molecular weight of ~200 kDa and spans the inner membrane (Capaldi 1982). This complex has 6-13 polypeptides, contains cytochromes *a* and a_3, and comprises two haem groups bound to different regions of the same large protein. It also has two copper ions, designated CuA and CuB (Babcock and Wikström 1992). This complex directs the electron flow from cytochrome *c* to O_2 to form H_2O (Babcock and Wikström 1992). The origin of the hydrogen ions involved in reaction (10) will be discussed subsequently. Cyanide inhibits complex IV and is termed a site III inhibitor (Vanden Hoek et al 1997). The reaction sequence of Complex IV is shown in equation (10).

$$4\text{Cyt } c^{2+} + 4H^+ + O_2 \longrightarrow 4\text{Cyt } c^{3+} + 2H_2O \qquad (10).$$

ATP synthase, also named F_oF_1-ATPase, has a molecular weight of 370-378 kDa and is approximately 8.5 nm in diameter and contains ~13-14 different polypeptide subunits (Walker et al 1991). ATP synthase is a large membrane protein complex and is located in the matrix side of the inner mitochondrial membrane (Racker et al 1965). There are two major components of this membrane complex, F_o and the roughly spherical complex F_1. A stalk connects the F_o and the F_1 components of ATP synthase (Depierre and Ernster 1977). The component F_o functions like the proton-specific channels of the complex in ATP synthase (Hatefi 1985). The biological role of F_1 is to catalyse condensation of ADP and P_i to form ATP (Figure 2.10).

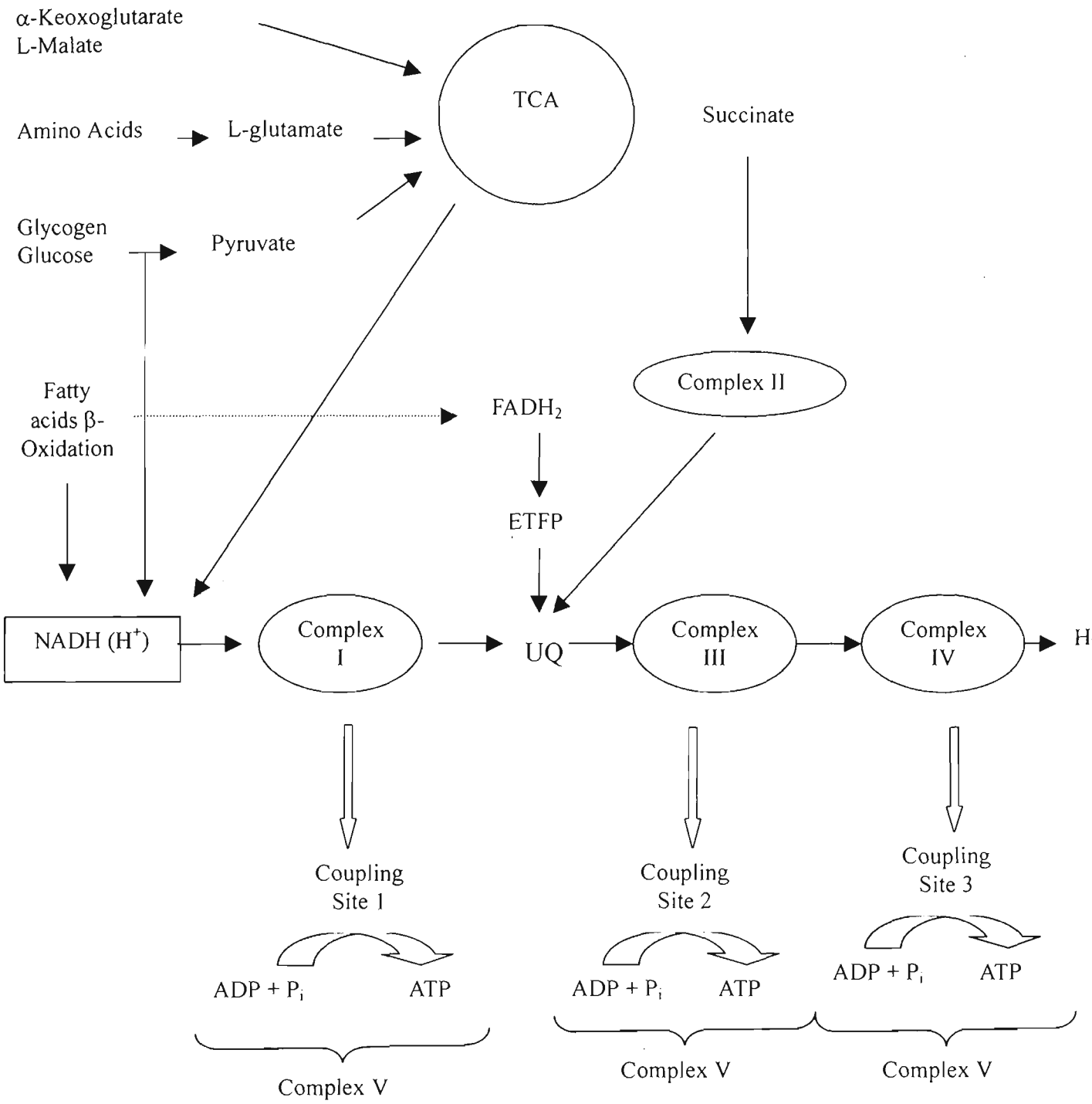


Figure 2.9. Oxidative Pathways of the substrates by the electron transfer chain in mitochondria.

TCA cycle, tricarboxylic acid cycle; UQ, ubiquinone (coenzyme Q); ETFP, the electron-transferring flavoprotein (ETFP); Coupling sites 1, 2 and 3 are the location where ATP synthesis is coupled to oxidoreduction steps and the free energy liberated at these sites. Complex V, ATP synthase.

2.2.2.5. The Chemiosmotic Theory and ATP Synthesis

The mechanism of ATP synthesis, is best explained by the chemiosmotic theory that was advanced by Mitchell (1961) (see Section 2.2.2.5). "*This theory has been accepted as one of the great unifying principles of twentieth century biology*" (Lehninger et al 1993). The chemiosmotic theory is generally accepted as the mechanism for mitochondrial oxidative phosphorylation. Transmembrane differences in proton concentration are central to energy transduction in this theory (Mitchell 1979). According to the chemiosmotic theory, ATP formation in mitochondria comprises several steps. Electron donors, such as, NADH + H^+ and $FADH_2$, supply electrons which as previously indicated are transferred along the respiratory chain. This process of electron transfer is associated with pumping protons across the inner mitochondrial membrane into the intermembrane space by Complexes I, III, and IV (Mitchell and Moyle 1967). Transferring electrons in the mitochondrial complexes occurs via a series of the oxidation-reduction reactions. It is the free energy change from these reactions which supplies energy for proton pumping in mitochondrial complexes (Mitchell 1961). With the release of protons into the intermembrane space a difference in proton concentration and hence electrical gradient across the inner mitochondrial membrane are produced. The negative membrane electrical potential in the matrix and the chemical gradient (pH) contribute to the formation of the proton-motive force. Subsequently, the proton-motive force drives the protons back into the mitochondrial matrix through ATP synthase. This movement provides the energy for the synthesis of ATP by the ATP synthase (Figure 2.10) and the process provides the link which couples the transfer of electrons to ATP synthesis (Mitchell 1961). As shown in Figure 2.9, the ECT complexes I, III and IV are the coupling sites. When one molecule of NADH + H^+ and one molecule $FADH_2$ are oxidized through the ETC and ATP synthase, $\bar{3}$ and 2 molecules of ATP are synthesized, respectively (Figure 2.9).

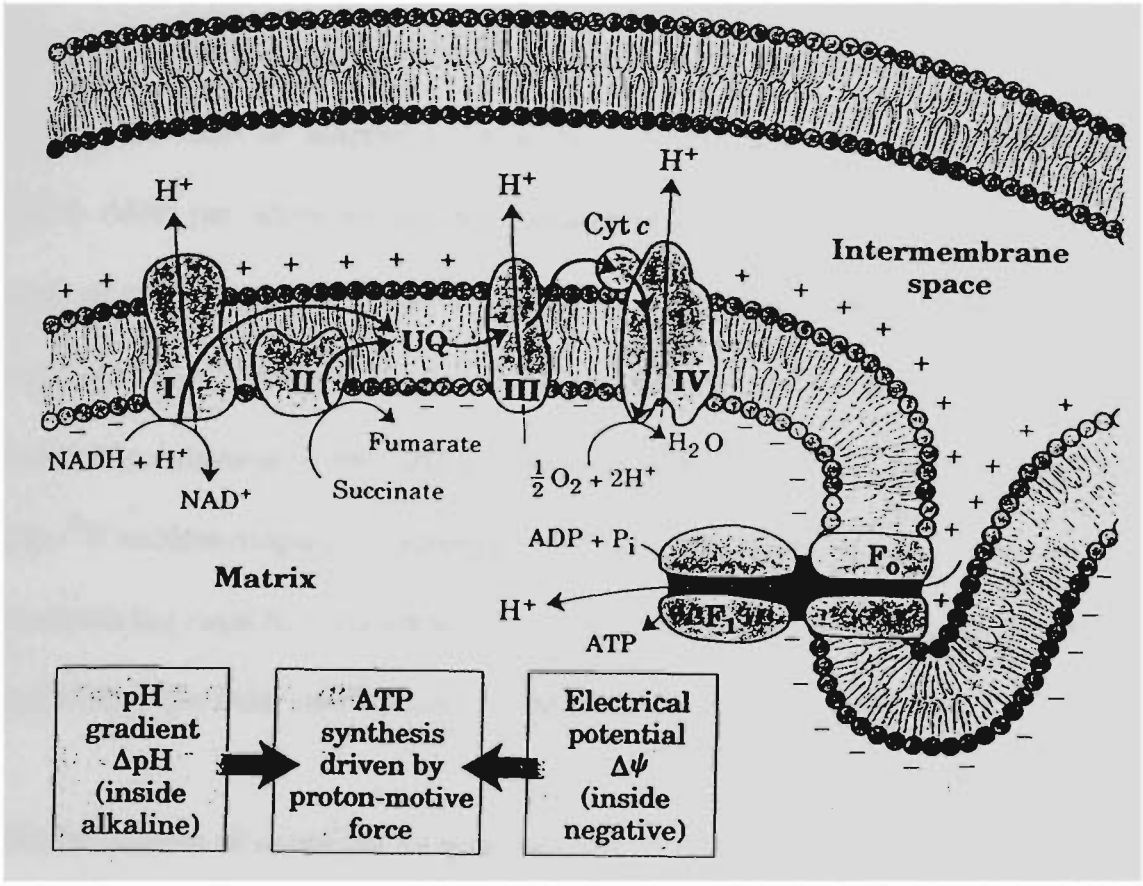


Figure 2.10. Schematic Diagram of the Chemiosmotic Theory

Electron flow is accompanied by proton transfer across the mitochondrial membrane, producing both a chemical (Δ pH) and an electrical ($\Delta\psi$) gradient. The inner mitochondrial membrane is impermeable to protons; protons can re-enter the matrix only through a proton-specific channel (F_o). The proton-motive force that drives protons back into the matrix provides the energy for ATP synthesis, catalysed by the F_1 complex associated with F_o (Lehninger et al 1993, Pp.559).

2.2.3. Assessment of Mitochondrial Function

A number of methods have been used to assess mitochondrial function. Examples of these methods include the determination of individual mitochondrial enzyme activity and the determination of mitochondrial oxygen consumption. Mitochondrial ATP synthesis by oxidative phosphorylation can be estimated *in vitro* through measuring the uptake rates of O_2 and calculating the ratio of number of molecules of inorganic phosphate (P_i) taken up to phosphorylate ADP per atom of oxygen consumed (P/O ratio or ADP/O ratio). Direct measurement of mitochondrial ATP production rate (MAPR) can be achieved *in vitro.* This method employed the luciferase enzyme which hydrolyzes ATP produced by mitochondria with luminometric detection of the light produced (Lundin et al 1976; Wibom et al 1990). In recent years, ^{31}P nuclear magnetic resonance (NMR) has also been employed *in vivo* to assess phosphate-containing muscle metabolites (Mancini et al 1989; Kutsuzawa et al 1992; 1995, Walker et al 1998). The basic mechanisms of the methods are described below.

2.2.3.1. Determination of oxidative enzyme activity

The different mitochondrial enzyme activities which have been used to assess mitochondrial function and aerobic capacity in skeletal muscle include:

- TCA cycle enzymes, such as SDH, CS (Essén and Henrikson 1980) and KGDH (Yeaman 1989).
- Fatty acid oxidative enzymes, such as HAD (Green et al 1984) and CPT I and CPT II (Mole et al 1971).
- GDH which is involved amino acid oxidation (Wibom et al 1992).
- The ETC enzymes, such as cytochrome *b5* reductase (Schantz and Källman 1989) cytochrome *c* oxidase (Soussi et al 1990).

Published data of enzyme activities including mitochondrial enzymes are highly variable.

This variability may be related to the different methods employed for determining enzyme activity, but may also be related the subjects age, sex and training status of subjects. Examples of the published CS and HAD activities data listed in Table 2.1. However, determination of muscle enzymes is still a common method and is accepted extensively to assay mitochondrial capacity or aerobic metabolism capacity.

Table 2.1. Variation of Enzyme Activities in Vastus Lateralis Muscle of Healthy Untrained Individuals

Reference	Unit	Age (year)/Sex	CS	HAD
Svedenhag et al 1984	μmol min^{-1} kg^{-1} w.w.	23/M	100	225
Green et al 1984	μmol min^{-1} g^{-1} w.w.	21/M	-	4.4±0.7
		20/F	-	4.5±0.9
Essén-Gustavsson & Borges 1986	μmol min^{-1} g^{-1} w.w.	20/M	28±3	22±3
		50/M	26±9	22±5
		20/F	27±3	23±4
		50/F	19±5	20±4
Tesch et al 1990	μmol min^{-1} g^{-1} w.w.	27/M	9.5±2	2.3±0.5
Wang et al 1993	μmol min^{-1} g^{-1} w. w.	20/F	7.41±1.4	13.8±3
Maltais et al 1996	μmol min^{-1} g^{-1} w.w.	54/M	36.8±8.6	5.5±1.4
Green et al 1991	μmol min^{-1} g^{-1} pro*	Young/M	48.9±4.4	40.8±3.6
Jakobsson et al 1995	μmol min^{-1} g^{-1} pro.	67/4M&2F	120.7±19*	59.4±9.3*
MacDougall et al 1998	μmol kg^{-1} pro h^{-1}	23/M	3.5±2	2.99±1.3

Values are expressed as mean ± SD. *Values are expressed as mean ± SEM. M, male; F, female; CS, citrate synthase; HAD, β-hydroxyacyl CoA dehydrogenase or L-3-hydroxyacyl CoA dehydrogenase, w.w., wet weight; m, muscle weight; pro, protein; h, hour.

2.2.3.2. Determination of Mitochondrial O_2 Consumption

Polargraphic determination of mitochondrial O_2 consumption is a common method to assess the oxidative capacity of mitochondria *in vitro*. The most common method employs the Clark electrode, which comprises a platinum cathode maintained at –0.8 V with respect to a silver anode by a polarizing voltage. This electrode is immersed in a solution of potassium chloride (KCl). An O_2-permeable Teflon membrane separates the KCl solution and a test medium. Therefore, the oxygen concentration in the KCl solution follows the alteration of oxygen concentration in the test medium. In this method, a small amount change of 0.5% O_2 concentration in test medium can be detected (Sherratt et al 1988). This method uses isolated mitochondria to measure mitochondrial oxygen consumption in the presence of substrates such as pyruvate, malate, succinate, and glutamate. The roles of these substrates in assessing mitochondrial function will be discussed in Section 2.2.3.3.

The mitochondrial respiratory chain utilises oxygen to synthesise ATP from ADP and Pi. The ratio of P/O quantitatively reflects the coupling of ATP production to electron transport (Kingsley-Hickman et al 1987). The number of moles of P_i or ADP consumed is equal to the number of moles of ATP synthesised. As indicated in Figure 2.8 and shown in equation 11, oxidation of one mole NADH gives a P/O ratio of 3. Similarly, $FADH_2$ gives a P/O of 2 in theory (see equation 11).

$$NADH + {}^1/_2O_2 + H^+ + 3ADP + 3Pi \leftrightarrow NAD^+ + H_2O + 3ATP. \qquad (11).$$

Practically, however, electron transfer is not completely coupled to ATP synthesis because protons in the mitochondrial intermembrane space may leak back into the matrix across the inner membrane directly, rather than though the ATP synthase (Mitchell 1961). Proton leak in mitochondria has been demonstrated in a number of tissues including skeletal muscle (Rolfe

and Brand 1996). Therefore, the number of ATP molecule synthesised may be less than the number of the theoretical P/O ratio. Lee et al (1996) have demonstrated that the P/O ratio which obtained from isolated rat liver mitochondria was 1.5 with succinate and 2.5 with NAD-linked substrates. The P/O ratio *in vitro* with pyruvate oxidation in skeletal muscle mitochondria was only ~0.83 (Rolfe and Brand 1996) indicating substantial proton leak. In mitochondria of brown adipose tissue, the protons leak into mitochondrial matrix via the uncoupling protein (UCP) or thermogenin and result in producing heat without ATP synthesis (Palou et al 1998). The UCP has also been found from other tissue mitochondria, for instance, UCP2 is widely expressed in adult human tissues, such as white adipose tissue (Fleury et al 1997) and UCP3 is found in skeletal muscle (Vidal-Puig 1997). Brand et al (1994), however, suggested that proton leak in mitochondria isolated from muscle tissue may not mediated by UCP.

Chance and Williams (1955) examined the kinetics of oxygen utilisation in a mitochondrial suspension using simultaneous measurements of oxygen consumption and NADH in the medium. They identified five states of respiration in mitochondria. Accordingly, State 1 is the initial optical density of the suspension. State 2 follows the addition of ADP to State 1. In this state the oxidation of NADH is produced by mitochondria utilising the endogenous substrate in the medium to oxidative phosphorylation of ADP. State 3 is the state of oxidative phosphorylation of ADP following the addition of substrate, such as -β-hydroxybutyrate. State 4 corresponds to the exhaustion of ADP and is verified by the coincident decrease in respiration as measured by a platinum microelectrode. State 5 occurs when the mitochondrial suspension becomes anaerobic. Therefore, mitochondrial ATP production by oxidative phosphorylation is undertaken in State 3. This state has been traditionally used in the assessment of mitochondrial oxidative function. The ratio of the respiratory rates in State 3 and State 4, so called the respiratory control ratio (RCR) or index (RCI), has been used as a standard for assessing the quality of suspensions of isolated mitochondria. The higher the

RCI, the better quality of the mitochondrial preparation (Wilson 1994). Generally, from good preparations of skeletal muscle, the values of RCI are 2.5 to 4 (Sherratt et al 1988).

Measuring mitochondrial O_2 consumption usually requires a large (0.5 to 5 g) muscle mass (Makinen and Lee 1968; Max et al 1972; Barth et al 1983). Recently, however, Madsen et al (1996) have developed a method using a small muscle sample (100-200 mg) obtained from a muscle needle biopsy, to measure mitochondrial O_2 consumption. Tonkonogi and Sahlin (1997) have demonstrated that this technique can quantitatively assess isolated mitochondrial respiration. The mitochondrial oxygen consumption was closely correlated to whole-body $\dot{V}O_2$ max. This method, however, dose not directly measure ATP production because O_2 consumption in mitochondria is uncoupled to ATP formation due to the mitochondrial proton leak. Rolfe et al (1999) demonstrated that the mitochondrial proton leak accounted for 34% of the respiration rate of the preparation of maximal tetanic contractions of the left gastrocnemius-soleus-plantaris muscle group in rats. Cortright et al (1999) demonstrated that the level of UCP-3/18s rRNA was significantly increased by 63% and 252% in red and white gastrocnemius, respectively, after acute exercise in rats. The UCP-3 gene expression was also increased by 331% in wither gastrocnemius of sedentary rats after muscle denervation. These indicated that the mitochondrial proton leak in skeletal muscle may increase after exercise and immobilisation. Other factors may also increase the mitochondrial proton leak, such as ischaemia (Borutaite et al 1995) and age (Harper et al 1998). Consequently, the mitochondrial oxygen consumption, in certain situations, may not completely reflect the total ATP formation in mitochondria.

2.2.3.3. Measurement of Mitochondrial ATP Production Rate (MAPR)

Mitochondrial ATP production rate (MAPR) is a method used for the assessment of mitochondrial function which employs a mitochondrial suspension. This method is based on the enzyme luciferase which hydrolyses ATP in the presence of its substrate luciferin. Isolated

mitochondria are incubated in a medium containing ADP, Pi, substrates, along with luciferin and luciferase (Wibom et al 1990). Luciferase catalyses the reaction of luciferin, O_2 and ATP, which is produced from mitochondria, to produce luminescence. The rate of light production is proportional to the ATP concentration over the range 10^{-11} to 10^{-6} mol L^{-1}. (Lundin et al 1976). This method directly determines mitochondrial ATP production (Wibom et al 1990). The reaction for the measurement of ATP using the luminescence method is shown in equation 12.

$$\text{ATP} + \text{Luciferin} + O_2 \xrightarrow{\text{Luciferase}} \text{Oxyluciferin} + \text{AMP} + PP_i + CO_2 + \text{light} \quad (12).$$

Consistent with the measurement of mitochondrial O_2 consumption, the analysis of MAPR need ADP, P_i and oxidative substrates. The use of various substrates can indicate the mitochondrial capacity for different metabolic functions. For example, substrates which are intermediates of carbohydrate and amino acid oxidation, such as pyruvate, malate, α-ketoglutarate and glutamate, can be used to determine the metabolic capacity of Complex I in the ETC of mitochondria. Palmitoyl-carnitine is used as a substrate to assess the oxidation of fatty acids in mitochondria. It is used to measure the capacity of CPTI, CPTII, β-oxidation and Complex III, but may also, at least in part, measured the function of Complex I (Figure 2.9). The reason for this is that fatty acid oxidation via β-oxidation produces $FADH_2$, which contributes a pair of electrons to the electron-transferring flavoprotein (ETFP). The ETFP is an inner membrane protein and is the immediate electron acceptor for all reduced acyl-CoA dehydrogenases in mitochondria which transfer the electrons to UQ (Frerman and Goodman 1985, Herrick et al 1994, see Figure 2.9). The process of fatty acid oxidation also produces NADH $+^{-}H^{+}$ which enters the ETC via Complex I for further oxidation. To determine mitochondrial Complex II function, succinate and rotenone are usually used together. As an ETC inhibitor, rotenone blocks the electron transfer from Complex I to UQ such that only

Complex II transfers electrons from succinate to UQ (see Figure 2.9). The combination of pyruvate, palmitoyl-carnitine, α-ketoglutarate and malate as substrates, used to measure MAPR, represent a combined capacity for fatty acid and carbohydrate utilization (Wibom et al 1992). Measurement of MAPR requires a much smaller sample of muscle (30-40 mg muscle) (Wibom et al 1990) than that which is required for measuring mitochondrial O_2 consumption (Madsen et al 1996). Therefore, measurement of MAPR is easily achieved using samples which are obtained by needle biopsy for studies of human skeletal muscle (Wibom and Hultman 1990; Wibom et al 1992; Berthon et al 1995). However, a disadvantage of both methods of MAPR and mitochondrial oxygen consumption are that they are performed *in vitro*.

2.2.3.4. Nuclei Magnetic Resonance (NMR) Spectroscopy

The nuclei of certain atoms such as hydrogen (^{1}H), carbon (^{13}C) and phosphorus (^{31}P) have net magnetic spin. Molecules containing such atoms can be observed using the process of NMR spectroscopy which can be used, therefore, in studies of phosphate-containing metabolites such as ATP, PCr and P_i in skeletal muscle (Walker et al 1998). Measurement of PCr and P_i in muscle can allow for calculation of the phosphocreatine ratio [PCr/(PCr + Pi)], which is an index of PCr concentration ([PCr]) (Stratton et al 1994). During recovery from exercise, the PCr resynthesis rate (d[PCr]/dt) reflects the rate of mitochondrial ATP synthesis (Stratton et al 1994). The ratio of PCr/P_i (Evans et al 1997) or ratio of P_i/PCr (Mancini et al 1989) is also used as indication of phosphorylation potential. NMR can be used to determine muscle pH and via muscle pH can estimate lactate accumulation in muscle (Massie et al 1988; Evan et al 1997). Measurement of muscle oxidative capacity or mitochondrial function using NMR is performed *in vivo*. The NMR method should be more objective, and more accurate as there are fewer factors to affect measurement compared with the methods of mitochondrial O_2 consumption and MAPR. This method, however, can not directly measure the absolute

amount of ATP production per unit muscle mass.

2.2.4. Summary

Mitochondrion in skeletal muscle consists of five functional components which are the outer membrane, the inner membrane, the intermembrane space, the cristae and the matrix. The main function of mitochondria is to use carbohydrate, fatty acid and amino acid as energy fuels to produce ATP. Through the TCA, β-oxidation and amino acid transamination, the energy fuels are degenerated into NADH + H^+ and $FADH_2$. The chemiosmotic theory for ATP synthesis is the best explanation of the process of ATP synthesis. According the theory, NADH and $FADH_2$ carry the electrons to the mitochondrial ETC. The electrons are transferred from Complexes I to IV associated with the oxidation-reduction reactions in these components of ETC. These reactions produce free energy changes which provided energy for proton pumping from the mitochondrial matrix into the mitochondrial intermembrane space. Following the proton pumping an electrical gradient and a chemical gradient (pH) across the inner mitochondrial membrane are produced. Subsequently, the protons across the inner mitochondrial membrane into the matrix and coupling ATP synthesis via ATP synthase. Several methods allow assessment of mitochondrial function, including measurement of mitochondrial enzyme activity, determination of mitochondrial O_2 consumption, determination of MAPR, and the ^{31}P NMR spectroscopy method.

2.3. FACTORS AFFECTING MITOCHONDRIAL CAPACITY

Skeletal muscle occupies about 30% of total body weight and utilises 40% of total oxygen in a resting state. Under certain kinds of physical activity, however, oxygen consumed by skeletal muscle may reach ~90% of total body oxygen consumption. Therefore, mitochondria,

play a pivotal role in the metabolism of energy in skeletal muscle. Mitochondria in skeletal muscle appear to be affected by a number of factors, for example, genders, age, muscle fibre type, physical activity, oxidants and calcium.

2.3.1. Gender

An ultrastructural study of the human vastus lateralis muscle has demonstrated gender differences in mitochondria between males and females (Hoppeler et al 1973). The volume density of the intermyofibrillar mitochondria was ~1.44-fold higher in males than females. In terms of mitochondrial enzymes, Green et al (1984) and Nygaard (1981) demonstrated that the activity of SDH in the vastus lateralis muscle was higher in males than females. For other mitochondrial enzymes, however, including HAD (Green, et al 1984), MDH (Bass et al 1976) and CS (Nyaard 1981), there were no differences between the two genders. Essén-Gustavsson and Borges (1986) demonstrated that CS activity in vastus lateralis muscle was significantly higher in men than women in 30 and 40 years age groups, but was lower in a group of men aged 60 years compared with women at same age. The enzyme activity of HAD, HK and LDH were similar between the women and men of age from 20 to 70 years. Hence, gender is a factor potentially influencing mitochondrial capacity in skeletal muscle.

2.3.2. Age

Mitochondria are altered histologically, biochemically and genetically with ageing. Marked histological changes with ageing include mitochondrial enlargement, matrix vacuolisation, shortened cristae and a loss in the density of granules (Wilson and Franks 1975; Miquel et al 1980). Orlander et al (1978) demonstrated that mitochondrial volume fraction in skeletal muscle significantly decreased in older (66-76 years) compared with younger (16-18 years)

subjects. A decline in mitochondrial O_2 consumption and the activity of Complexes I and IV has been demonstrated in aged groups (Cooper et al 1992). Trounce et al (1989) examined the State III mitochondrial respiration with various substrates in isolated muscle mitochondria in 29 subjects aged 16-92 years. They found that mitochondrial respiratory rate with all substrates was significantly negatively correlated with age. Rooyackers et al (1996) demonstrated that the fractional rate of muscle mitochondrial protein synthesis was significantly lower in the middle aged group (mean 54 years) and in the older aged group (mean 73 years) compared with young individuals (mean 24 years). These changes in mitochondrial function may explain a decline in human maximal aerobic capacity of about 9-15% per 10 years (Kasch 1976). Impaired capacity of oxidative phosphorylation in mitochondria of skeletal muscle with ageing has been linked to oxygen free radicals and other oxidants (Adelman et al 1988; Hayakawa et al 1991; Sohal and Dubey.1994; Ames et al 1995). The effects of oxygen free radicals on mitochondria will be discussed in Section 2.3.5.

2.3.3. Skeletal Muscle Fibre Types

Human skeletal muscle comprises two major fibre types, type I and II. Type II is further subclassified into type IIa and IIb when using histochemical procedures (Brooke and Kaiser 1970; Saltin et al 1977). Further more, type Ic and IIc and IIab muscle fibres are also identified in humans using histochemical methods (Wang et al 1993; Staron 1994). Alternately, using immunochemical and electrophoretic techniques several different fibre types of myosin heavy chains (MHC) can be identified. The MHC are named type MHCI, MHCIIa, MHCIIb, and MHCIIc (Draeger et al 1987; Staron and Pette 1990). Recently, MHCIIx (Sant'Ana Pereira et al 1996; Pette and Staron 1997; Carroll et al 1998) or MHCIId/x (Pette and Staron 1997) was identified, as a fast-twitch fibres, replacing to MCHb in human skeletal muscle. Fry et al (1994) demonstrated that the fibre I, IIa and IIb proportions

(histochemical method) strong correlated with the proportion of MHCI, MCHIIa and MCHIIb in vastus lateralis muscle, respectively ($r > 0.83$, $P < 0.05$). Therefore, the MHCI, MHCIIa and MHCIIb are equivalnt to type I, IIa and IIb fibres, respectively. Nevertheless, in the present study, since muscle fibre types were identified as type I, IIa and IIb fibres using histochemical methods, this terminology is used subsequently in this thesis.

In general, type I fibres exhibit high aerobic capacity by virtue of their rich mitochondrial content (Staron et al 1984, Wang et al 1993) and show high levels of oxidative enzyme activity (Essén et al 1975; Essén and Henrikson 1980). They also show a high content of myoglobin and lipid stores (Staron et al 1984). These fibres are known as slow twitch and as fatigue resistant fibres (Garnett et al 1978; Brooke and Kaiser 1970). They also have abundant capillarisation (Anderson 1975). Type I fibres are therefore slow-twitch oxidative fibres. Type IIb fibres have fast contraction velocities and fewer mitochondria, and tend to be better suited for anaerobic metabolism because they have increased glycolytic capabilities (Lowry et al 1978), and are hence called fast-twitch glycolytic fibres. Type IIa fibres are termed fast-twitch oxidative fibres and intermediate in most respects between the type I and type IIb fibres (Brooke and Kaiser 1970; Clamann and Broecker 1979; Thompson 1994). The mitochondrial characteristics, mitochondrial enzyme activities, muscle fibre capillaries and glycolytic enzyme activities in the skeletal muscle fibre types in the human are shown in Table 2.2.

It has been demonstrated that the muscle fibre type composition and area are different between the individuals with different physical training statues. Untrained healthy subjects have approximately 50% of each muscle fibre type in vastus lateralis muscle (Costill et al 1976). In the same muscle, endurance-trained athletes have a high proportion of type I fibres (up to 80%) (Baumann et al 1987) and sprint trained athletes, have a high proportion of type II fibres (up to 73%) (Costill et al 1976).

Endurance training can increase the proportion of type I fibres by 12% and reduce the proportion of type IIb fibres by 24% (Howald et al 1985). Resistance-trained subjects show an

increase in the cross-sectional areas in all fibres (type I & II) of muscle (McCall et al 1996), which is more pronounced in type II fibres (Green et al 1998). In addition, resistance-trained subjects have shown a lower proportion of type IIb fibres (-33 to -90%) (Schantz and Källman 1989; Staron et al 1991; Wang et al 1993; Staron et al 1994; Kraemer et al 1995; Green et al 1998) and an highter (27% to 74%) proportion of type IIa fibres (Staron et al 1991; Kraemer et al 1995) in an number of different skeletal muscles. Studies of myosin heavy chain isoform contents have also demonstrated that resistance training induces muscle fibre transformation from type IIb to IIa fibres (Adams et al 1993; Fry et al 1993; Carroll et al 1998).

Table 2.2. Mitochondrial and Glycolytic Capacities and Capillarity in Fibre Types of Human Vastus Lateralis Muscle

	Type I	Type IIa	Type IIb	Ref.
Oxidative capacity	High	moderate	low	
Mitochondrial volume percentage	3.04±0.94	2.25±0.75	1.62±0.55.	4**[a]
(%)	7.77±0.27	5.89±0.29	5.35±0.35	5*[a]
Mitochondrial Size (μm^2)	0.133±0.003	0.126±0.004	0.119±0.004	5*[a]
Mitochondrial number/μm^2	0.50±0.02	0.40±0.01	0.38±0.02	5*[a]
Number of capillaries around fibre	4.9±0.3	4.5±0.3	3.5±0.4	1**[b]
Number of capillaries around fibre type per fibre type area $10^{-3}(\mu m)^{-2}$	1.0±0.03	0.79±0.02	0.76±0.03	1**[b]
3-Hydroxyacyl-CoA dehydrogenase (HAD)	14.8	11.6	7.1	3*[a]
Succinate dehydrogenase (SDH)	2.5	1.9	1.0	2*[a]
	7.1	4.8	2.5	3*[a]
Citrate synthase (CS)	10.8	8.6	6.5	3*[a]
Phosphorylase (PHOSPH)	2.8	5. 8	8.8	3*[a]
Phosphofructokinase (PFK)	3.9	5.6	6.9	2*[b]
Lactate dehydrogenase (LDH)	59	221	293	3*[a]

Values are expressed by mean and mean ± SD. Data source: (1) Andersen (1975). (2). Essén et al 1975. (3) Essén and Henrikson (1980). (4) Staron et al (1984). (5) Wang et al (1993). Enzyme activity value expressed by $\mu mol\ min^{-1}\ g^{-1}$ w.w. * $P < 0.05$ ** $P < 0.01$. a, different among the three fibre types; b, different between the type IIb and type I or type II.

Muscle immobilisation, however, can induce muscle fibre atrophy in type I fibres, and decrease the proportion of type I fibres from 42.3% to 37.2% (Halkjær-Kristensen and Ingemann-Hansen 1985a). Similarly, certain diseases are associated with changes in muscle fibre type proportions. For example, Sullivan et al (1990) reported that the proportion of type I fibres of vastus lateralis was 36% in a group of patients with congestive heart failure (CHF). Jakobsson et al (1990) also found that a group of patients with chronic obstructive pulmonary disease (COPD) had only 17% of type I fibre proportion in the vastus lateralis.

2.3.4. Physical Activity

The effects of different types of physical activity on the mitochondrial capacity of skeletal muscle have been widely investigated. Muscle immobilisation, disuse and detraining result in a reduction of mitochondrial function. Endurance training results in enhanced mitochondrial capacity. The effects of resistance training on mitochondrial capacity, however, are uncertain.

2.3.4.1. Muscle Disuse, Immobilisation and Detraining

Most studies have demonstrated that immobilisation or disuse in both animals and humans can impair skeletal muscle mitochondria. In cats for example, mitochondrial morphological degeneration in skeletal muscle was observed after 6 weeks of immobilisation (Cooper and Iowa 1972). Muscle mitochondrial O_2 consumption in rats was reduced after only one day (Max 1972) or two days of hindlimb immobilisation (Krieger et al 1980). Similarly, Rifenberick and Max (1974) reported that the capacity of muscle mitochondria to oxidise glucose, pyruvate, palmitate, and β-hydroxybutyrate was reduced with immobilisation in rats' limbs. Yajid et al (1998) demonstrated that the gastrocnemius muscle mitochondrial State 3 respiration of intermyofibrillar mitochondria and subsarcolemmal mitochondria, with pyruvate + malate, was decreased by 59% and 18%, respectively, after 4 weeks hindlimb

Côté et al (1988) demonstrated that the activity of the enzymes MDH, HAD, and KGDH were significantly increased after repeated concentric isokinetic strength training for ten weeks. Wang et al (1993) also showed that 20 weeks resistance training resulted in an increase in the activity of cytochrome c oxidase. They, however, demonstrate there were no changes in the enzyme activity of CK, CS, HAD and PFK & PHOSPH. Similarly, Schantz and Källman (1989) found that there were no differences in enzyme activity of CS, MDH, HAD, glycerol-3-phosphate dehydrogenase (GPDH), aspartate aminotrasferase (ASAT), cytochrome b_5 reductase, and PFK in skeletal muscles between the subjects, who had been strength trained, for 8.6 years, and the untrained controls. In contrast, Tesch et al (1987) demonstrated that the activity of CS and other metabolic enzymes including PFK, HK, creatine kinase (CK), myokinase (MK), and myofibrillar ATPase in skeletal muscle all decreased after 6 months of strength training. Examples of effects of resistance training on muscle mitochondrial enzyme are showing in Table 2.3. Enzyme activity in different muscle fibres of resistance–trained subjects has a similar pattern as the endurance-trained (Essén-Gustavsson and Henriksson 1984) and untrained subjects (Essén et al 1975). That is a higher activity of oxidative enzymes (CS and HAD) in type I fibres and higher activity of LDH in type II fibres (Tesch et al 1989).

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

These conflicting findings in mitochondrial capacity after resistance training may be due to differences in training styles, with varied workloads, training intensity and duration of resistance training. Details of the effects of resistance training on skeletal muscle size, mitochondrial volume and enzyme activities from previous studies are listed in Table 2.3. Where resistance training induces muscle fibre hypertrophy this may be an important factor influencing the assessment of mitochondrial capacity when these values of mitochondrial respiration and enzyme activity are expressed per unit muscle weight. In some instance, therefore, changes in mitochondrial volume and/or mitochondrial enzyme activities could be masked by greater increase in muscle mass (hypertrophy). For example, in a 12 weeks

resistance training study by Komi et al (1978) and 7 weeks study by Costill et al (1979) no evidence of muscle hypertrophy observed. In both of the studies, however, significant increases in muscle mitochondrial enzymes MDH and SDH were reported. By contrast, the studies by MacDougllas et al (1979; 1982) involving 6 months heavy resistance upper body training showed increased the CSA of all muscle fibre types reported. In both these studies, however, both the mitochondrial volume density and ratio of mitochondrial volume density to myofibrilar volume density were decreased. In general, it can be seen from Table 2.3, where resistance training results in muscle hypertrophy muscle mitochondrial volume density and oxidative enzyme activities are either unchanged or decreased.

Table 2.3. The Details of Effects of Resistance Training on Muscle Fibre Type and Oxidative Capacity

Sources	Age (year) /Sex	Muscle	Training	CSA Changes (%)				Enzyme Activity Changes (%)						Mitochondrial Chan (%)	
				I	IIa	IIb	TM	MDH	SDH	CS	KGDH	HAD	COX	VD	V/M
Komi et al (1978)	14/F&M	VL	12w				↔	↑27*	↑32*						
Costill et al (1979)	23/M	VL	7w.	↔	↔	↔		↑14*	↑11*						
MacDougall et al (1979)	22/M	TB	6mo.	ST ↑27*	FT ↑33*									↓26*	↓25
MacDougall et al (1982)	22/M	TB	6mo	ST ↑9*	FT ↑17*									↓11*	↓14
Staron et al (1984)‡	24/M	VL	> 3 yrs.	↑12*	↑33*		↑9*							↓* 35 in I, IIa & IIb	
Lüthi et al (1986)	18/M	VL	6w.				↑8							↑9	↔
Tesch et al (1987)	26/M	VL	6mo.	ST ↑4	FT ↑29*		↑16*			↔					
Alway et al (1988)	17-32/M	TS	10.6 yrs.	↑*	↑*									↓36** in I & ↓29** in II	
Côté et al (1988)	24/M&F	VL	10w.	↔	↔	↔	↔	↑15*			↑89*	↑25*			
Schantz & Kallman (1989)	25/M	DM	8.6 yrs.	□	↑48*	↑68*	↑34*	↔		↔		↔	↔		
Wang et al (1993)	20/F	VL	18w	↑20*	↑29*	↑22*				↔		↔	↑28*	↑18*, 24* & 6 in I, IIa & IIb	↔
				↑20*	↑25*	↑6	(AV)								
Green et al (1998)	19/M	VL	12w.	↑14*	↑24*				↔						

Changes = (RT - Controls)/controls × 100. AV, absolute volume; DM, detoid muscle; TB, Triceps brachii; TS, triceps surae; TM, thigh muscle; V, volume; V/M, volume/myofibrils ratio; VD, volume density; VL, Vastus latralis. ↑, increased; ↓, decreased; ↔, unchanged;* $P < 0.05$.

2.3.4.4. Effect of Muscle Fatigue on Mitochondria

Muscle fatigue has been defined by the National Heart, Lung, and Blood Institute (NHLBI) in 1990, as an inability to maintain the required or expected force or power output, resulting from muscle activity under load, reversible by rest. Little is known about mitochondrial changes in muscle fatigue. Gollnick and King (1969) found marked swelling of muscle mitochondria isolated from rats which were run to exhaustion. Nimmo and Snow (1982) confirmed the swollen mitochondria following fatigue exercise. In horses exercised to fatigue, the muscle mitochondrial area increased 3-4 fold at exercise intensities from 40% to 100% of the $\dot{V}O_2$max (McCutcheon et al 1992). A depression in the respiratory capacity of mitochondria has also been reported after high-intensity exercise to exhaustion in rats (Dohm et al 1972; 1975) and horses (Gollnick et al 1990).

The mechanism by which mitochondrial respiration was depressed in muscle fatigue is unknown. Muscle acidosis may impair oxidative metabolism (Gollnick et al 1990). During exercise, increased muscle temperature could disrupt mitochondrial structure and impair mitochondrial function (Brooks et al 1971; Willis and Jackman 1994). The over production of free radicals in muscle during exercise also showed a capacity to damage mitochondrial lipid membranes and impair their function (Davies et al 1982; Barclay and Hansel 1991). In contrast, Tonkonogi et al (1999) demonstrated that mitochondrial ATP production and respiratory index were not changed in human muscle biopsies during higher-intensity intermittent cycling exercise (130% of $\dot{V}O_2$max). They also shown that the maximal ADP - stinulated respiration in skinned fibre was increased by 23% from rest to exhaustion and remained higher at 110 minutes recovery samples. The study of Tonkonogi et al (1999) indicated that mitochondrial function is not impaired, but improved during high intense fatigue exercise. Endurance training may reduce the mitochondrial damage of skeletal muscle in fatigue. Terjung et al (1972) found that endurance-trained rats exercised to exhaustion

showed no change in the capacity of rat mitochondria to oxidise substrates (pyruvate plus malate) and generate ATP. Madsen et al (1996) demonstrated that the skeletal muscle mitochondrial RCI in moderately endurance trained men was elevated, with a slightly increased mitochondrial State 3 and decreased State 4 respiration after prolonged exhaustive exercise. The effects of resistance training and strength training on mitochondrial function in fatigue is unknown.

2.3.5. Effects of Oxidants and Antioxidants on Mitochondria

2.3.5.1. Free Radicals, Oxidants and Reactive Oxygen Species

Free radicals contain an unpaired electron or several unpaired electrons in the outer electron orbit of an atom or molecule. The unpaired electron is exchangeable. The main free radicals include superoxide anion ($O_2^{\bullet}$), hydroperoxyl radical ($HO_2^{\bullet}$), and hydroxyl radical ($^{\bullet}OH$). The term, reactive oxygen species (ROS) is not synonymous with free radicals. ROS represent a broader spectrum of species including nonradicals derivatives of oxygen (Sen 1995), for example, hydrogen peroxide (H_2O_2). In addition to the above free radicals, ROS also include alkyl radical (R•) (R• an abbreviation for organic molecules in general), peroxyl radical (ROO•) and hydroperoxide (ROOH) (Karlsson 1997). The term of oxidants may have the same meaning as ROS (Fehér et al 1987; Packer 1997), and is also including products of lipid peroxidation, protein oxidation and nucleic acids oxidation (Packer 1997). Examples of oxidants are lipid oxidant malondialdehyde (MDA) from lipid hydroperoxide (Janero 1990), protein carbonyl compounds from protein oxidation (Stadtman and Oliver 1991) and 8-hydroxy-2'-deoxyguanosine, a marker of DNA damage (Shigenaga et al 1989) . In addition, nitric oxide (NO) is also an inorganic free-radical gaseous molecule because NO contains an unpaired electron in an outer orbital (Jenkins 1988). Oxidants, such as oxygen-derived free

radicals, lipid peroxidation products, oxidation products of protein and nucleic acids have been recognised as important adverse products on strenuous physical exercise (Davies et al 1982; Barclay and Hansel 1991; Sen et al 1994) as well as being implicated in pathological conditions recently, such as CyA-induced oxidative stress, resulting in liver cytotoxicity (Wolf et al 1997) and renal failure (Baliga et al 1997).

Under physiological conditions the mitochondrial ETC generates minimal free radicals and the cellular antioxidant systems control the oxidants well (Ji 1995). In certain circumstances, such as strenuous exercise (Davies et al 1982; Barclay and Hansel 1991; Sen 1994), ischaemia-reperfusion (Kawasaki et al 1993; Novelli et al 1997) or hypoxia–reoxygenation (Damerau et al 1993), however, oxidants are increased significantly. Packer (1997) proposed a number of possibilities for production of oxidants during strenuous exercise. One possibility is electron 'leak' in mitochondrial ETC to produce superoxide radicals. Since whole-body oxygen consumption and oxygen flux in muscle fibres increases during exercise, the electron leak in ETC may also increase to produce more free radicals. Another possibility is ischaemia-reperfusion in organs and tissues. During exercise some organs and tissues, such as kidneys, may experience hypoxia due to blood flow being shunted away from these organs to the working muscle. It is possible also that working muscle may become hypoxia when working muscle energy demands are beyond the O_2 supply. Once exercise cease, these organs and tissues obtain blood via reperfusion and re-oxygenation which may lead to a burst of ROS production. Vanden Hoek et al (1997) exposed cardiomyocytes to the mitochondrial ETC inhibitors rotenone, antimycin and cyanide which are known to act at sites I, II and III, of the ETC, respectively (see Section 2.2.2.4). These cells were treated with a regime designed to imitate ischaemia-reperfusion. They demonstrated that these inhibitors significantly increased H_2O_2 and hydroxyl radicals with the order of cyanide > antimycin > rotenone > controls. This experiment indicates the inhibitors acting at the more distal ETC carriers resulted in more oxidant generation. They also showed that antioxidants (2-mercaptopropionylglycine,

phenanthroline) and lowering O_2 levels in the reperfusion which is a 'antioxidant' strategy can improve the cells viability, contractility and reduced rate of cells death during the cyanide exposure. Accordingly, Vanden Hoek et al (1997) concluded that mitochondrial that the ETC is the source of oxidant injury which is greatest when the mitochondrial ETC reaches a fully reduced redox state and is exposed to oxygen.

2.3.5.2. Effect of Free Radicals and Oxidants on Mitochondria

Zhang et al (1990) have demonstrated that the free radicals $^{\bullet}OH$, and $O_2^{\bullet}$ alone or in combination can rapidly inactivate enzymes of the mitochondrial ETC in bovine heart submitochondrial particles. These enzymes included NADH dehydrogenase, NADH oxidase, succinate dehydrogenase, succinate oxidase, and ATPase. Soussi et al (1990) observed that a ~40% reduction of cytochrome *c* oxidase activity occurred in rat skeletal muscle after ischaemia and reperfusion. A number of studies investigating oxidant inactivation of mitochondrial functions have demonstrated that oxidants may damage the inner mitochondrial membrane (Chia et al 1983; De Groot et al 1985). Lipid peroxidation may impair mitochondrial membrane electrochemical potential and lead to ATP depletion (Carini et al 1992). Oxidants may also disturb the mitochondrial metabolism of proteins (Stadtman 1992; Sohal and Dubey 1994), cellular DNA and mtDNA (Adelman et al 1988; Cortopassi and Arnheim 1990; Fraga 1990), and lipids (Ames et al 1995).

2.3.5.3. Antioxidant Protection of Mitochondria

Antioxidants include endogenous physiological defence systems and exogenous antioxidants. The endogenous defence systems serve to remove ROS. The exogenous antioxidants, such as vitamin E (Sumida et al 1989) can detoxify the ROS. The major endogenous antioxidants are the enzymes superoxide dismutase (SOD), catalase (Cat), and glutathione peroxidase (GPX). SOD converts $O_2^{\bullet}$ into O_2 and H_2O_2. The enzymes, Cat and GPX, then convert H_2O_2 to form

H_2O and O_2 (Sen 1995). Antioxidants, such as vitamin E, may have a protective effect against exercise-induced skeletal muscle oxidative damage (Sumida et al 1989; Ji 1995). It has also been observed that vitamin E can protect human skeletal muscle ischaemia-reperfusion damage. Novelli et al (1997) demonstrated that vitamin E significantly reduced the content of malondialdehyde (MDA) which is a catabolite of the polyunsaturated fatty acid peroxidation, the mitochondrial swelling and intermyofibrillar oedema in human skeletal muscle after surgical ischaemia-reperfusion. Coenzyme Q_{10} is also thought to act as an antioxidant (Frei et al 1990). Coenzyme Q_{10} may be a recycler of vitamin E (Kagan et al 1990) and be able to prevent oxidative damage of membranes by hydroperoxide-induced lipid peroxidation (Leibovitz et al 1990). The $^{\bullet}OH$ scavenger mannitol and SOD (Zhang et al 1990) can inhibit the $^{\bullet}OH$ and $O_2^{\bullet}$-dependent inactivation of mitochondrial electron transport components and ATPase *in vitro*.

2.3.6. Effect of Calcium on Mitochondria

Calcium is an intracellular messenger in many eukaryotic signal-transferring processes, for example, vision and the regulation of muscle contraction. It is also deeply involved with physiological function in mitochondria and the pathological alteration of mitochondria such as in ischaemic heart disease (Ferrari 1996).

2.3.6.1. Physiological Effects of Calcium on Mitochondria

When Ca^{2+} concentration ([Ca2$^+$]) is elevated in the cytosol, Ca^{2+} can be transferred through the inner mitochondrial membrane into the matrix. Following an increase of [Ca^{2+}] in the matrix, the multiple Ca^{2+}-dependent oxidative enzymes, which are located in the mitochondria, can be activated. There are at least four important dehydrogenases dependent on [Ca^{2+}] in mitochondria. They are PDH (Denton et al 1972), the NAD+-linked isocitranate

dehydrogenase (Denton et al 1978), α-ketoglutarate-dehydrogenase or 2-oxoglutarate-dehydrogenase complex (KGDH or OGDH) (McCormack and Denton 1979) and the HAD (Hansford and Chappell 1967). Consequently, these enzymes may enhance rates of mitochondrial NADH formation and ATP synthesis (Denton and McCormack 1985; McMillin and Madden 1989). Ca^{2+} may act as a regulator of the adenine nucleotide translocator function and directly activates ATP/ADP carrier. Ca^{2+} also regulates the mitochondrial oxidative phosphorylation during muscle contraction (Madsen et al 1996; Korzeniewski 1998). In addition, mitochondrial can contribute to the buffering of extra-mitochondrial Ca^{2+} concentration at 1-3 μmol L^{-1}, to regulate the $[Ca^{2+}]$ in cytoplasm (Nicholls 1978).

2.3.6.2. Functional Damage of Mitochondria Induced by Calcium Overload

Damage from hypoxia-reoxygenation and ischemia-reperfusion has been related to Ca^{2+} overload in the cell and mitochondria (Lochner et al 1987; Darley-Usmar et al 1990). This may involve an impaired mitochondrial ATP production during either ischaemia or reperfusion situations (Ferrari 1996). Damage to mitochondrial function associated with mitochondrial Ca^{2+} overload has been linked to impairment of the ETC components (Darley-Usmar et al 1990; Veitch et al 1992) and decreased mitochondrial oxidative phosphorylation (Tuena De Gomes-Puvou et al 1980; Ferrari 1996). The reperfusion of isolated heart 90 minutes of ischaemia, results in increased free radicals, severe disruption of oxidative phosphorylation capacity and mitochondrial Ca^{2+} accumulation (Ferrari 1996). These changes may be the result of an opening of the mitochondrial permeability transition pore (MPTP) in the inner mitochondrial membrane (Hunter and Haworth 1979).

The MPTP is a minimal ~2 to 2.8 nm in diameter (Massari and Azzone 1972; Crompton and Costi 1990), and allows diffusion of solutes with molecular masses up to 1200 daltons (Gunter and Pfeiffer 1990). Ca^{2+} at 0.5 μmol L^{-1} (Altschuld et al 1992), P_i at 0.5 mmol L^{-1} (Petronilli et al 1993), adenine nucleotide depletion and oxidative stress can open the pore

(Crompton and Costi 1988; Gunter and Pfeiffer 1990; Savage et al 1991). Any breakdown of inner membrane potential of the mitochondria also promotes the pore opening (Bernardi 1992). The pore opening, however, is reversible with the removal of Ca^{2+} (Crompton and Costi 1990). ATP and ADP can block the pore (Haworth and Hunter 1980; Richter et al 1990). Complete pore blockade may require more than 1 mmol L^{-1} ATP (Duchen et al 1993). Cyclosporine A (CyA) has also been used as the mitochondrial pore blocker (Altschuld et al 1992). Recently, Fontaine et al (1998) reveal that skeletal muscle mitochondria also have MPTP, but the pore open is related to the rate of electron flow through Complex I rather than regulated by membrane potential, matrix pH, Ca^{2+} uptake and production of H_2O_2. Their study indicated that the MPTP in skeletal muscle may be different to the MPTP in other organs, such as heart and liver.

It has been demonstrated that cellular cytosolic [Ca^{2+}] and mitochondrial matrix [Ca^{2+}] increases are one of the key features in cellular damage and necrosis under many non-physiological conditions. Examples of these conditions include oxidant stress/oxygen free radical damage (Richter et al 1995), hypoxia-reoxygenation (Darley-Usmar et al 1990; Tanaka et al 1998) or ischaemia-reperfusion injury (Crompton 1990; Steenbergen et al 1990). When [Ca^{2+}] increased in mitochondria, the MPTP in the inner mitochondrial membrane is opened and results in massive swelling of mitochondria (Crompton 1990) associated with a reduction of ATP production (Tanaka et al 1998). Mitochondrial damage caused by Ca^{2+} overload also induces activation of protease and endonuclease, the oxidation of protein thiols, ionic disturbances, preoxidation of the membrane, the hydrolysis of phospholipid, and the ultimate disruption of the mitochondrial membrane and DNA fragmentation (Orrenius et al 1989, see Figure 2.11).

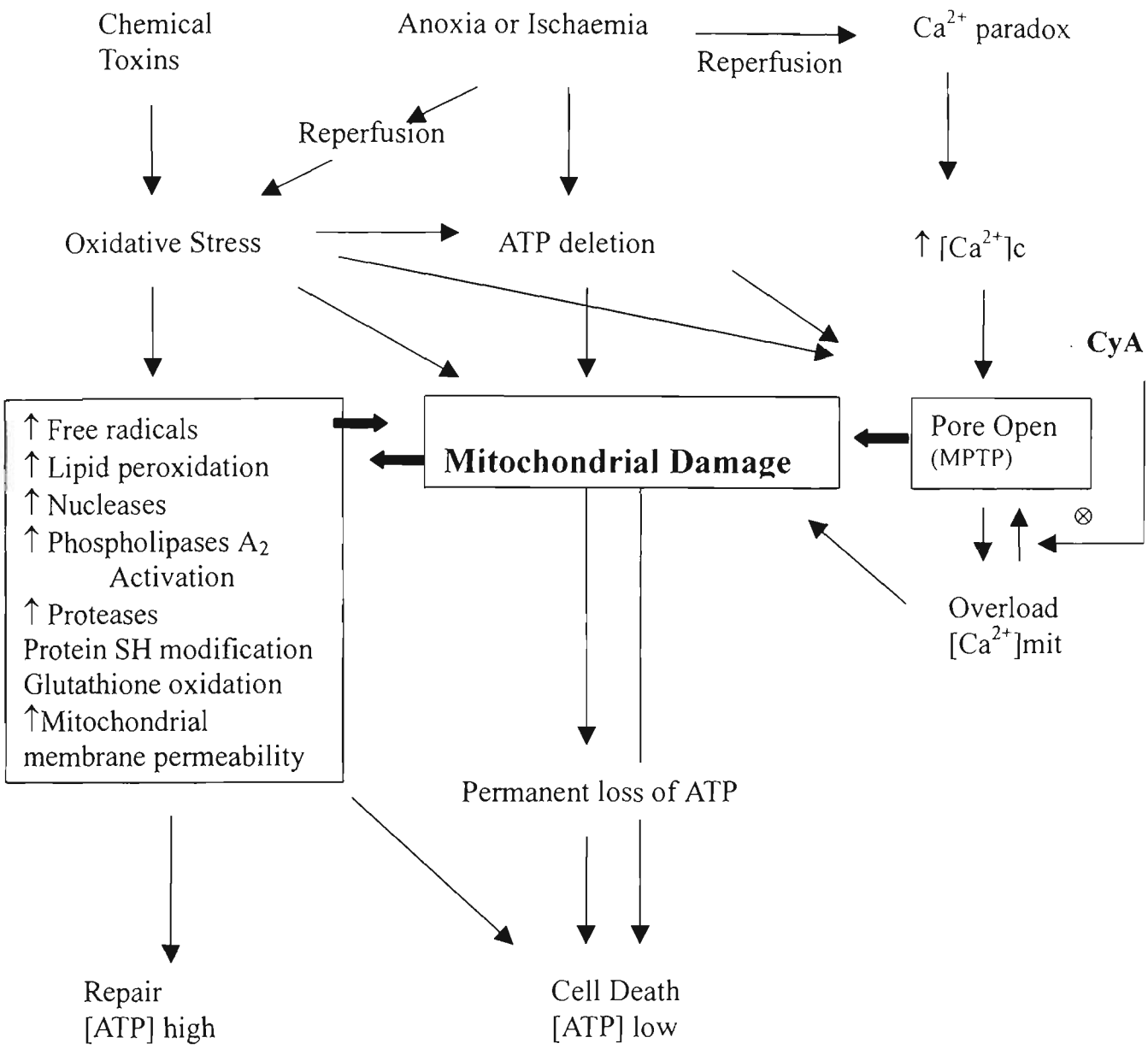


Figure 2.11. Proposed Relations of Oxygen Stress, Anoxia & Ischaemia and Reperfusion, and Pore Opening

Scheme is modified based on Halestrap (1994). CyA, Cyclosporine A; c, cytoplasma; mit, mitochondria; MPT, mitochondrial permeability transition; $[Ca^{2+}]c$, cytoplasm calcium concentration; $[Ca^{2+}]m$, mitochondrial calcium concentration; [ATP], ATP concentration.

2.3.7. Summary

Mitochondrial function in skeletal muscle can be affected by a number of factors. Skeletal muscle type I fibres have a much higher mitochondrial capacity compared with type II fibres. Mitochondrial volumes in males' muscle are higher than in females, but most mitochondrial enzyme activities are the same between the both genders. Ageing, not only appears to reduce the mitochondrial capacity of oxidative phosphorylation in human skeletal muscle, but also produces mitochondrial morphological degeneration. The depression of mitochondrial function, associated with ageing, has been linked to mitochondrial damage caused by free radicals and oxidants. Different levels of physical activity as shown in exercise training and immobilisation also affect mitochondrial capacity. Skeletal muscle disuse, immobilisation, long term bed rest and detraining can all reduce mitochondrial oxidative phosphorylation capacity and enzyme activities. Mitochondrial morphological degeneration also occurs after a period of immobilisation. In contrast, endurance training enhances mitochondrial capacity in skeletal muscle. The effects of resistance training on mitochondrial capacity in muscle is unclear. Previous studies have shown conflicting results, in which mitochondrial volume and mitochondrial enzymes have been shown to be increased, decreased and to remain unchanged. During exercise, muscle fatigue may reduce muscle mitochondrial function which may be caused by acidosis, heat and increased oxidant production. The increased oxidants and free radicals can inactivate enzymes in the mitochondrial ETC and can damage the mitochondrial membrane as well. These oxidants can also disturb mitochondrial protein and DNA metabolism. On the other hand, the endogenous (e.g. SOD and Cat) and exogenous (e.g. vitamin E) antioxidants can remove oxidants and protect mitochondria. Calcium has a significant effects on mitochondria. Increase in mitochondrial [Ca^{2+}] can activate some mitochondrial enzymes to enhance mitochondrial function, whereas Ca^{2+} overload in the matrix of mitochondria can induce mitochondrial damage both morphologically and functionally.

2.4. OUTCOMES, EXERCISE LIMITATION AND POTENTIAL CAUSES POST HEART-LUNG AND LUNG TRANSPLANTATION

During the last 15 years, heart-lung transplantation (HLTx) and lung transplantation (LTx) have become an established modes of treatment for many end-stage heart-lung and pulmonary diseases. Techniques presently utilised include HLTx (Reitz et al 1982), SLTx (Toronto lung transplant group 1986), bilateral lung transplantation (BLTx) or DLTx (Patterson et al 1988). The BLTx or DLTx is either performed as bilateral sequential lung transplantation or infrequently en-block double lung with bronchial revascularisation (Svendsen et al 1995). More recently, live donor bilateral lobar transplantation has been reported (Couetil et al 1997).

2.4.1. Heart-Lung and Lung Transplantation Indications

According to the 1997, the Registry of the International Society for Heart and Lung Transplantation (RISHLT) official report (Hosenpud et al 1997), the three most common operative indications for HLTx are congenital heart diseases (CHD) (29.8%), primary pulmonary hypertension (PPH) (26.9%) and cystic fibrosis (CF) (15.6%). The two most common indications for DLTx are CF (33.8%) and emphysema (17.2%). The top two indications of SLTx are emphysema (44.3%) and idiopathic pulmonary fibrosis (IPF) (20.1%). Hence, chronic obstructive pulmonary diseases (COPD) are the dominant lung transplant operative indications.

2.4.2. Post-transplantation Outcomes and Exercise Limitation

For the recipients, HLTx and LTx result in improvement of survival, lung function, quality of life and exercise capacity following successful transplantation (Dawkins et al 1985; Williams

et al 1990; 1992a; Cooper et al 1994a). After transplantation, the capacity for physical activity in these patients improves dramatically during the first few months, but the exercise capacity remains lower than normal or predicted levels when they recovered from their operation without clinical complications (Williams and Snell 1997). Incremental exercise tests on these recipients are characterized by low peak exercise workrate, low $\dot{V}O_2$ peak or $\dot{V}O_2$ max (Banner et al 1989; Orens et al 1995; Williams 1992a; Levine et al 1994) and an low lactate threshold (LT) (Levy et al 1993; Ross et al 1993).

2.4.2.1. Survival

Candidates for HLTx and LTx have experienced irreversible, progressive, end-stage cardiac and pulmonary disease. The life expectancy of these patients is estimated to be less than 12 to 18 months (Davis and Pasque 1995). Generally, actuarial survival post-transplantation in HLTx is ~60% in 1 year, and the half-life expectancy for those survivors after the first year (also called the conditional half-life) is 8.3 years (Hosenpud et al 1997). The survival rate during the first year post-transplantation in DLTx and SLTx is similar at ~60%. The half-life expectancy in DLTx and SLTx is 4.5 and 3.7 years, respectively (Hosenpud et al 1997). Therefore, HLTx and LTx significantly improve of the survival rate of patients with end-stage heart and lung diseases.

2.4.2.2. Quality of Life

The quality of life (QOL) of long-term survivors of post HLTx and LTx shows significant improvement (Dennis et al 1993; Spray 1993; Busschbach et al 1994; Cooper et al 1994b). Gross et al (1995) used a self-report questionnaire which included the Medical Outcomes of Health Survey (MOS-20) to compared a group of adult lung transplant candidates and a group of adult lung transplant recipients. MOS-20 is used to assess a number of dimensions of QOL including the physical, role and social function, mental health, health perceptions and bodily

pain. The results demonstrated that adult recipients of LTx reported higher levels of happiness, more satisfaction with their life (79%) and health (76%) compared with adult LTx candidates. According to the follow-up information of LTx in the USA (Hosenpud et al 1997), approximately 78.9% and 85.7% LTx patients experienced no limitation in physical activity during the first year and in the first two years post-transplantation, respectively. Around 23.1% and 10.4% of LTx patients in the first year and about 30.2% and 10.6% of LTx patients in the first two years engaged in full-time and part-time work, respectively. There were about 45.9% and 57.8% of LTx recipients that were hospitalised during the first year of follow-up and the first two years follow-up, respectively.

2.4.2.3. Pulmonary Haemodynamic Change

The reduction of pulmonary hypertension and improvement of right ventricular function are important for the recovery of cardiovascular function in SLTx recipients. A number of follow-up studies in SLTx have demonstrated a dramatic decrease in pulmonary hypertension and pulmonary vascular resistance post-transplantation (De Hoyos et al 1992; Bando et al 1994; Kramer et al 1994; Pasque et al 1995; Bjørtuft et al 1996). These changes have also been associated with a remarkable improvement in right ventricular function (Carere et al 1991) and a reduction of right ventricular hypertrophic mass (Ritchie et al 1993; Kramer et al 1994; Frist et al 1995). The pulmonary hemodynaemic and cardiac disorders may persist in some LTx patients (Pasque et al 1995) and these may be the central factor which limit exercise capacity in these patients (see Section 2.4.3.1).

2.4.2.4. Pulmonary Function

Lung transplantation markedly enhances pulmonary function in the recipients. Spirometry indicates that each of the forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), minute ventilation ($\dot{V}E$), diffusion capacity (DLco) and arterial O_2 partial

pressure (PaO_2) are significantly improved, after HLTx (Theodore et al 1992), DLTx and SLTx (Williams et al 1990; 1992a; Levy et al1993; Ross et al 1993; Levine et al 1994; Bando et al 1995). In the first few months after operation, HLTx and DLTx recipients have only shown mild to moderate restrictive ventilatory defects during spirometry tests. Spirometry testing in HLTx recipients gradually improved to normal after the first few months (Morrison et al 1992). In DLTx, however, a mild restriction ventilation persisted when these recipients were free of clinical complications (Bando et al 1995). SLTx recipients have shown persistent abnormalities of ventilation in spirometry tests because the native lung of SLTx recipients still functioned with the lung allograft. The native lung in SLTx patients often presents with impairment of function due to previous disease. This results in an FEV_1, FVC and DLco in SLTx patients that only are a level of ~50%, ~60% and ~60% of predicted values, respectively (Williams et al 1990; Levy et al 1993; Bando et al 1995). Ventilation and perfusion lung scans in SLTx demonstrated that lung ventilation/perfusion was mismatched due to the dead space ventilation in the native lung (Grossman et al 1990; Bando et al 1995; Pasque et al 1995). The mild impaired pulmonary function seems not the factor to limit exercise capacity in HLTx and DLTx recipients. Pulmonary ventilation/perfusion mismatch, however, may be a factor of exercise limitation in SLTx (see Section 2.4.3.1).

2.4.2.5. Exercise Performance

The exercise performance in HLTx and LTx patients has been assessed by a number of tests. These include the 6-minute walk test (Williams et al 1990), modified Bruce protocol treadmill test or Stage 1 exercise test (Williams et al 1992), and formal cardiopulmonary exercise test (Orens et al1993). The exercise performance is significantly improved within the first 3 to 6 months after operation in patients with HLTx (Ambrosino et al 1996), DLTx (Williams et al 1990; Cooper et al 1994a) and SLTx (Williams et al 1990; Orens et al 1995). Recovery exercise performance in these patients has reached a plateaus after 6 to 12 months in HLTx

and LTx patients. In the second year, the capacity of exercise only improved slightly in HLTx and LTx patients (Williams et al 1990; 1992). In incremental maximal exercise testing, both HLTx and LTx (DLTx and SLTx) recipients demonstrated a low peak workrate associated with lower $\dot{V}O_2$peak, lower HRpeak and lower LT compared with normal controls or predicted values (William et al 1992; Levy 1993; Howard et al 1994). Peak oxygen uptake ($\dot{V}O_2$max) in these patients moderately decreased ranging from 45 to 60% of the predicted value (Miyoshi 1990; Theodore et al 1992; Williams 1992a; 1992b; Levy et al 1993). This result was remarkably similar among HLTx, DLTx and SLTx recipients (Howard et al 1994; Orens et al 1995; Williams and Snell 1997). The abnormality of exercise performance also appears in those patients in which pulmonary ventilation and cardiovascular function had been improved to near normal levels after transplantation (Madden et al 1992; Bando et al 1995). In addition, Ambrosino et al (1996) investigated the peak torque of isokinetic contractions in flexor (hamstring) and extensor (quadriceps) muscles of the thigh in a group of HLTx patients. They demonstrated a significant improvement in the peak torque of isokinetic contraction in both the hamstring quadriceps muscles of leg at 6 months post-operation. These muscle functions were improved with the time but were still less than the normal after 18 months.

2.4.2.6. Abnormalities in Exercise Metabolism

Post-transplant, most HLTx and DLTx recipients have normal arterial blood gases with normal pulmonary arterial and alveolar O_2 gradients ($P_{A\text{-}a}O_2$ gradients) (Burke et al 1986; Williams et al 1990; 1992a; Bando et al 1995). These patients do not exhibit blood oxygen desaturation during exercise (Williams et al 1992a; 1992b). The SLTx receptions, however, experience mild blood oxygen denaturation, with normocapnoea and a widened $P_{A\text{-}a}O_2$ gradients during intense exercise near their $\dot{V}O_2$peak (Bando et al 1995; Mal et al 1994) During incremental exercise, both HLTx and LTx have an early rise in blood lactate

concentration (Williams et al 1992a; Ross et al 1993; Levy et al 1993; Howard et al 1994). More recently, Evans et al (1997) used ^{31}P MRS to investigate oxidative capacity of the quadriceps muscle in LTx patients. They demonstrated that the resting muscle pH was significantly lower in LTx than the controls. This study also showed that the lactate threshold in LTx was lower during submaximal exercise compared with the controls. Information relating to skeletal muscle metabolism in LTx, however, remains inadequate.

2.4.3. Potential Causes of Exercise Limitation In LTx

The exercise limitation in HLTx and LTx patients may be potentially caused by central defects in heart and lung or by peripheral defects in skeletal muscle, which are indicated below.

2.4.3.1. Central Defects

Heart and lung function abnormalities are the central defects, for example, heart failure, pulmonary inflammation and pulmonary ventilation and circulation disturbances (Howard et al 1994). A number of causes result in central defects and these are discussed below.

Denervated Allograft

HLTx recipients have a denervated heart and lung allografts. There has been in considerable debate over the last decade regarding the adequacy of the response in cardiac allograft to exercise (Savin et al 1980; Kavanagh et al 1988; Rudas et al 1992). Jensen et al (1991) demonstrated that heart transplant recipients had a normal function of allografts and an adequate oxygen delivery during incremental exercise testing. Kimoff et al (1990) investigated the ventilatory response in HLTx patients in progressive incremental exercise. They found that CO_2 output ($\dot{V}CO_2$) in HLTx patients was higher than controls, because the ventilatory frequency was slightly increased in HLTx patients. Otherwise, all parameters of pulmonary function in HLTx were similar to controls. These patients also had similar

ventilation at peak exercise compared with the controls. Sciurba et al (1988) demonstrated that exercise capacity and pulmonary function in HLTx patients were similar with heart transplant patients. The two group patients have a appropriate levels of ventilation during rest and maximal exercise. Accordingly, these mild ventilation alterations in HLTx patients do not appear to limit exercise capacity in HLTx and DLTx (Sciurba et al 1988; Levy et al 1993).

Acute Rejection and Infection

Most acute allograft rejection occurs in the first three months. Organ biopsy confirmed a rejection rate of 60% to 70% in the first month post HLTx and LTx (De Hoyos et al 1992; Cooper et al 1994a). Bacterial and other fungal and viral infections are the most common complication and cause of death after the first month up to the twelfth month post HLTx and LTx (Hosenpud et al 1997). Acute lung rejection and pulmonary infection results in lung inflammatory infiltration which leads to a reduction of the gas diffusive capacity in the allograft. This would reduce exercise capacity significantly, since the pathological changes of pulmonary infection, such as fever and hypoxaemia are inevitable outcomes in these patients. Once sepsis occurs, one of the most severe metabolic consequences is loss of body protein. The main source of the lost protein is from skeletal muscle protein breakdown, especially, degradation of myofibrillar protein (Hasselgren et al 1989; Hasselgren and Fischer 1997). A reduction of skeletal muscle protein synthesis and inhibited uptake of amino acids also occurs in sepsis (Hasselgren et al 1986). These would potentially cause skeletal muscle wasting with a loss of muscle mass and increased susceptibility to fatigue.

Chronic Rejection and Obliterans Bronchiolitis

The development of chronic allograft dysfunction is the main central defect post-lung transplantation. This has been characterised by decreased FEV_1 (Cooper et al 1993) and inflammation and occlusion of airways (Snell et al 1997). The latter pathological changes in bronchioles is so called obliterans bronchiolitis (OB). In chronic allograft dysfunction, OB is the most common histological finding (Cooper et al 1993), but the physiological

manifestation of chronic allograft dysfunction often does not correlate with the histological findings of lung biopsy (Davis and Pasque 1995). One year after transplantation, OB is the most common cause of mortality in HLTx and LTx (Sarris et al 1994; Hosenpud et al 1997). There are a number of possible etiologies for OB. Chronic rejection and bacterial or a variety of other infections, such as cytomegalovirus (CMV) and the influenza virus are thought to be possible etiological causes on the small airways (Burke et al 1984; Winter et al 1994; Kramer et al 1996; Kroshus et al 1997). Burke et al (1984) suggested that lung denervation and loss of bronchial circulation might play a role in damaging the small airway resulting in bronchiolitis. Immunologically mediated responses targeted at the small airways may be another potential cause (Reichenspurner et al 1995). Human lymphocyte antigen (HLA) mismatch and autoimmune diseases may also be important factors (Burke et al 1987; Kroshus et al 1997). Patients with primary pulmonary hypertension before transplantation have been found to be more susceptible to OB (Kshettry et al 1996). The transplant patients with OB have a progressive deterioration in pulmonary function (Patterson et al 1996) and they may be associated with abnormalities in skeletal muscle and limit the exercise capacity, but there are no reports so far.

Lung Ventilation/Perfusion Mismatching

As mentioned in Section 2.4.2, pulmonary ventilation/perfusion mismatching is another central defect affecting pulmonary oxygen exchange particularly in SLTx (Grossman 1990). Ventilation/perfusion mismatching in HLTx and DLTx, may suggest subtle abnormalities in the allograft such as acute rejection, subclinical rejection, and the alteration of pulmonary circulation by drugs, inducing systemic vascular contraction and chronic rejection or OB (Howard 1994). Ventilation/perfusion mismatching in lung can produce hypoxaemia which is a potential factor to limit exercise capacity in LTx patients.

Cardiac Defects

DLTx, and SLTx recipients had a similar cardiac performance response to the exercise tests

revealing a low HRpeak compared with controls or predicted values in maximal exercise (Howard et al 1994; Levy et al 1993). Ross et al (1993) reported that the maximal heart rate response to peak exercise between pre and post LTx was similar, but the cardiac index (CI) (CI = cardiac output per unit body surface area, L min^{-1} m^{-2}) significantly improved post-transplantation. Williams and Snell (1997) indicate that cardiac function is not the predominant factor limiting exercise if the cardiac function is normal. On the other hand, some patients may have pulmonary hypertension, left and right ventricular function incompetence, specially in the SLTx patients with pulmonary hypertension (HP) (Pasque et al 1995). In these patients cardiac incompetence may reduce exercise capacity (Ross et al 1993). Similarly, cardiac incompetence may also occur in some long-term survivors of HLTx because chronic heart rejection may cause coronary vascular occlusive disease (Scott et al 1992).

2.4.3.2. Peripheral Defect in Skeletal Muscle

Since skeletal muscle is the biggest peripheral tissue and its function is vital for physical activity in humans. In recent years, peripheral skeletal muscle defect has been regarded as the most important factor limiting exercise performance in LTx patients (Howard et al 1994; Williams and Snell 1997). The aetiology of peripheral defect in skeletal muscle is multi-factorial and can occur either pre-operation or post-operation.

Pre-Transplant Skeletal Muscle Injury

Patients who require HLTx and LTx have all suffered with severe chronic pulmonary and/or heart disease, which are so called end-stage lung and/or heart diseases. The response of skeletal muscle to long-term bed rest, severe respiratory dysfunction and congestive heart failure most often leads to muscle deconditioning because muscle disuse, hypoxaemia and/or an inadequate blood circulation in peripheral skeletal muscle can result in muscle debilitation.

Skeletal Muscle Deconditioning. As previously indicated, long-term bed rest, muscle disuse,

and immobilisation are all associated with loss of skeletal muscle mass, strength and reduced skeletal muscle mitochondrial mass or volume. These individuals have muscle atrophy and fibre type alteration, and a low $\dot{V}O_2$ max and limited exercise performance (see Section 2.3.1). This alteration in muscle is commonly referred to as physical deconditioning. Skeletal muscle deconditioning in certain diseases, such as CHF and pulmonary disease, may also involve a series of pathological processes. However, it is difficult to distinguish between physical and pathological deconditioning in these patients because patients with pathological disorders are also potentially susceptible to physical deconditioning. Therefore, this sort of muscle deconditioning may have both physical and pathological causes. The pathological changes of deconditioning in cardiac and pulmonary disease are discussed below. Kayanakis (1989) has defined that muscle deconditioning syndrome in heart failure patients has two causes: reduction of the nutritive blood flow in skeletal muscle and specific alteration of mitochondrial oxidative metabolism.

Skeletal Muscle alterations in Cardiovascular Diseases. A large number of studies have shown skeletal muscle impairment in CHF. The major alterations in skeletal muscle histology is muscle fibres atrophy associated with a significantly lower proportion of muscle type I fibres (~22%-~45%) and a higher proportion of type II (~55% to ~78%) or type IIb (~22%-~33%) fibres compared with normal controls (Mancini et al 1989, Sullivan et al 1990; Massie et al 1996). In the muscle fibres, capillary length density (Drexler et al 1992) and number of capillaries (Sullivan et al 1990) are significantly lower by -20% and -18% (type I) to -24% (type IIa) in CHF patients than in the normal controls, respectively. The capillary reduction may result in diminished blood flow in muscle (Wilson et al 1984). Drexler et al (1992) also demonstrated that the patients with CHF have significantly lower mitochondria volume density (-18%) and lower surface density of cristae mitochondria (-17%) in muscle fibre compared with the controls. Reduction in mitochondrial function is also evidence by lower oxidative enzyme activity (Massie et al 1988; Chati et al 1994; 1996; Massie et al 1996;

Hambrecht et al 1997). For instance, CS activity in skeletal muscle was 26.7 μmol L^{-1} in patients with CHF and 43 μmol L^{-1} in controls (Sullivan et al 1990). More important, patients with CHF have a poor exercise performance (Minotti et al 1992; Chati et al 1994; 1996; Massie et al 1996).

Skeletal Muscle Alteration in Pulmonary Disease. Respiratory function dysfunction in lung diseases can affect skeletal muscle. Hamilton et al (1995) measured peripheral muscle strength in 4,617 subjects. Peripheral muscle strength was lower in patients with angina and cardiac disease compared with the healthy controls ($P < 0.001$), such as, work of knee extension was 46 kg in angina patients, 45.2 kg in patients with cardiac disease and 50 kg in controls. The muscle strength in patients with pulmonary disease and patients with combined pulmonary and cardiac diseases was significantly lower than controls, angina patients and patients with cardiac disease ($P < 0.001$). For example, work of knee extension was 41 kg in patients with pulmonary disease and was 40.5 kg in patients with combined pulmonary and cardiac diseases. The patients with angina, cardiac disease, pulmonary disease and with combined pulmonary and cardiac diseases also had a lower maximal work capacity compared with normal controls (971 kpm min^{-1} or 102% of predicted value) by 77.3%, 84%, 72% and 70% of predicted levels, respectively. Therefore, impaired cardiao-pulmonary functionsare an important factor leading to reduction of muscle function.

Skeletal muscle fibre types were also changed in patients with pulmonary disease. Jakobsson et al (1990) showed that skeletal muscle fibre-type changes in COPD. The COPD patients with respiratory failure had only 17% of type I fibres and patients without respiratory failure had 22.3% of type I fibres. The proportion of type I fibre had a positive correlation with arterial partial oxygen pressure (PO_2) in these patients. Whittom et al (1998) also found that a group of patients with COPD had a lesser proportion of type I fibres (34% in COPD *vs.* ~58% in controls), higher proportion of type IIb fibres (15% in COPD *vs.* 5% in controls). The muscle area was smaller in type I (-40%) and type IIa fibres (-29%) in vastus lateralis muscle

compared with controls.

Patients with COPD also exhibited low activity of mitochondrial enzymes. Maltais et al (1996) showed that patients with COPD had a lower muscle CS activity (22.8 *vs.* 36.8 μmol min^{-1} g^{-1}) and HAD activities (3.1 *vs.* 5.5 μmol min^{-1} g^{-1}) compared with controls. Jakobsson et al (1995) also showed that patients with COPD had a 29% lower CS activity in skeletal muscle compared with the normal control subjects (85.3 *vs.* 120.7 μmol min^{-1} g^{-1} protein). Payen et al (1993) have demonstrated that the capacity of oxidative phosphorylation in skeletal muscle was low in patients with COPD and chronic hypoxaemia. The muscle oxidative capacity of these patients was measured in calf muscle by ^{31}P-NMR before and after foot exercise. In resting muscle, COPD patients had 14% lower level of ratio of ATP/(PCr + Pi + phosphomonoesters) compared with controls indicating reduced mitochondrial function in the patients. Patients also exhibited a lower intracellular pH (-5%) and higher Pi/PCr ratio (870%) at end of exercise. The half time for PCr resynthesis during recovery was greater by 122% in the patients compared with controls. These data reflect a reduced oxidative capacity and greater reliance on anaerobic metabolism during exercise. It is significant, therefore, Jakobsson et al (1995) demonstrated that COPD patients had 34% greater level of the glycolytic enzyme PFK compared with controls.

Fiaccadori et al (1987) demonstrated that a group patients with COPD had a lower intracellular pH (6.77 in COPD *vs.* 7.03 in controls, $P < 0.05$). These patients also had lower ATP (16.8 *vs.* 23.8 mmol L^{-1}), PCr (49 *vs.* 76.9 mmol L^{-1}), ATP/ADP ratio (5.14 *vs.* 8.36) and increased Cr (66.5 *vs.* 51.8 mmol L^{-1}) and lactate concentration (15.4 *vs.* 8.6 mmol kg^{-1}) in resting quadriceps femoris muscle compared with healthy controls. The study of Jakobsson and Jorfeldt (1995) also showed that muscle ATP and ratio of PCr/(PCr + Cr) were significantly lower in COPD patients than in healthy controls by -15% (16.3 *vs.* 19.1 mmol kg^{-1}) and -24% (0.39 *vs.* 0.51), respectively. In addition, Pouw et al (1998) investigated

skeletal muscle metabolism in two group patients with COPD. One group patients had lower (56% of predicted value) lung diffusion capacity for carbon monoxide (DLco) and other group COPD patients had a near normal lung DLco (76% of predicted value). Muscle IMP concentration in the patients with low DLco, was significantly increased (0.18 mmol kg^{-1}dw), but muscle IMP did not change in the COPD patients with near normal DLco lung function (0.06 mmol kg^{-1}). The DLco between the two groups was significantly different ($P < 0.05$). The ratio of PCr/Cr in resting muscle in this group of COPD patients was 10% lower than the control patients. Hypoxaemia may be an important factor leading to metabolic deterioration in patients with pulmonary disease because long-term oxygen therapy or oxygen supplementation improved muscle metabolism (Payen 1993; Jakobsson et al 1995). Hence, in heart and lung transplant candidates, cardiovascular system failure or pulmonary system dysfunction can result in skeletal muscle deconditioning which consists of alteration of muscle fibre, reduction of mitochondrial oxidative capacity and metabolic abnormalities in skeletal muscle.

Post-Operative Factors

After transplantation, heart-lung and LTx patients may still exist skeletal muscle deconditioning. The immunosuppressive drugs taken may also result in peripheral defects, especially, corticosteroids and CyA.

Persistent Deconditioning in Skeletal Muscle. Previous studies have suggested that exercise intolerance associated with low $\dot{V}O_2$ peak in HLTx and LTx recipients during exercise may be caused by persistent deconditioning post-transplantation (Miyoshi et al 1990; Williams 1992a; Ross et al 1993; Levine et al 1994). A number of studies have demonstrated the low exercise capacity persistence after heart-lung and lung transplantation (Ross et al 1993; Ambrosino et al 1996). Skeletal muscle abnormalities in HLTx and LTx patients may also persist after operation, in spite of the fact that cardiopulmonary diseases have been relieved or partially relieved by successful organ transplantation. Ambrosino et al (1996) demonstrated that HLTx

recipients have a low peak torque during isokinetic knee contractions in the leg flexor-hamstring muscle (IFX) and in the extensor-quadriceps muscle (LEX). LFX and LEX peak torques in these HLTx patients were 28 and 48 Newton-meter (Nm), respectively, when they were admitted to hospital for heart-lung transplantation. These patients also associated with a low $\dot{V}O_2$ peak (~40% of predicted value) at that time. Post-transplantation, the LFX and LEX peak torques were significantly increased to 35 and 86 Nm, respectively, after 6 months rehabilitation training. These parameters were still lower than predicted values (LFX ~ 60% and LEX ~ 70% predicted values). $\dot{V}O_2$ peak, however, did not significantly alter during the 18 months observation. The maximal level of $\dot{V}O_2$ peak in these patients was ~56% of predicted value at 6 months post operation. Evans et al (1997) confirmed in ^{31}P-MRS study that there is an abnormal oxidative capacity in skeletal muscle of LTx recipients 5 to 38 months after transplantation. Patients in this study showed a greater level of acidosis in muscle at rest (intracellular pH 7.07 *vs.* 7.12 unit) compared with the controls. During incremental quadriceps exercise test, the patients exercise for 5.4 minutes compared with 7.8 minutes for controls. Muscle pH fell during exercise, at a mean oxygen consumption level of 282 ml min^{-1} compared with a a significantly higher level of 577 ml min^{-1} in the controls. The alterations in muscle metabolism in the lung transplant recipients observed by Evans et al (1997), are indicative of a lower skeletal muscle oxidative capacity and a greater reliance on anaerobic metabolism in muscle at rest and during exercise. Bussières et al (1997) investigated skeletal muscle morphology and biochemistry for cardiac transplant recipients. They found that the proportion of type II fibres was 66% pre-operation and this did not alter one year after operation. The cross-sectional area of three type muscle fibres significantly increased (from type I ~3,119 to ~4,250, IIa ~2,770 to ~3,700 and IIb ~2,256 to ~2,900 μm^2, $P < 0.05$) after operation 12 months. These muscle fibre areas, however, were still lower than published normal values (type I ~4,980 - ~6,800, IIa ~5,000 - ~6,400 and IIb ~3.200 - ~5.700 μm^2; Andersen 1975; Sullivan et al 1990; Whittom et al 1998). The activity of muscle

enzymes PFK, CS and HAD increased by 26%, 47% and 63%, respectively, 12 months after operation. The patients in the study by Bussières et al (1997), however, still had low peak $\dot{V}O_2$ uptake (46% and 60% predicted value in post operation 3 and 12 months, respectively) during incremental exercise tests. There deficiencies were still evident, even if these patients participated in a physical rehabilitation program in the first 3 months and further continue exercise training at home post-operation. Therefore, the skeletal muscle deconditioning which occurred before operation in heart transplantation persists post-transplantation. It is possible, therefore, a similar outcome may occur in LTx patients.

Immunosuppressive Treatment. Post-transplantation, patients undertake long-term immunosuppressive drug treatment. These drugs have different side effects which may impact adversely on skeletal muscle and exercise intolerance.

Corticosteroids. Animal studies have shown that corticosteroids can inhibit protein synthesis in rat skeletal muscle (Odedra et al 1983). Severe wasting of respiratory muscle and limb muscle with a corresponding loss of body weight and muscle weight have been shown in triamcinolone and methylprednisolone (synthetic glucocorticoid) treated rats (both 80 mg kg^{-1} day × 5 days injection) (Nava et al 1996). These two steroids significantly induced type IIb fibre atrophy in the diaphragm, scalenus medius and gastrocnemius muscle. Prednisolone (PNL) at 5mg kg^{-1} dosage, however, did not cause muscle fibre atrophy (Dekhuijzen et al 1995). Type IIa fibre atrophy in the diaphragm was only observed in the triamcinolone group (Nava et al 1996). Consistently, Wilcox et al (1989) demonstrated that corticosteroids mainly target skeletal muscle type II fibres (atrophy) rather than type I fibres. Lettéron et al (1997) investigated the effect of dexamethasone, one of glucocorticoids (20 μmol L^{-1}), on hepatic mitochondrial function and lipid metabolism in mice. They have demonstrated that dexamethasone can inhibit mitochondrial matrix fatty acid enzyme activity *in vitro* and *in vivo*. They also demonstrated that the steroid can significantly inhibit isolated mice hepatic mitochondrial State III respiration indicated by a lower RCR and lower ADP/O ratio in

presence of the substrates malate plus glutamate. A reduction of RCR and ADP/O ratio in presence of succinate as substrate were also demonstrated in the study of Lettéron et al (1997). Their study indicates that glucocorticoids can inhibit the function of Complex I and Complex II in mitochondrial ETC and reduce mitochondrial ATP production capacity *in vitro*. Similarly, Simon et al (1997) have also demonstrated that PNL (1 μmol L^{-1}) decreased the respiratory control ratio (-13.4%) with succinate and rotenone showing impairment of the ATP synthesis in rat kidney mitochondria. In contrast, Peter et al (1970) injected triamcinolone (5mg kg^{-1} day) to group of rats for ten days, then isolated mitochondria form gastrocnemius muscle for determining mitochondrial oxidative function. Their study did not shown any effect of triamcinolone *in vitro* on the mitochondrial respiration in presence of substrate combination of pyruvate + malate, succinate + rotenone and ascorbate + *N'N'N'N'*-tetramethyl-*p*-phenylenediamine (TMPD).

Acute myopathy has been reported following high doses of corticosteroids or long-term steroid therapy (Panegyres et al 1993; Al-Lozi et al 1994; Decramer et al 1996; Hanson et al 1997). Van Balkom et al (1997) demonstrated that corticosteroid-treated (6 mg kg^{-1} PNL for 3 weeks) rat diaphragm muscle reduced the maximal isometric force and slowed the maximal shortening velocity by ~20%. Peak power output of the corticosteroid-treated diaphragm muscle was only 60% of controls. Decramer et al (1996) observed a group of steroid-induced myopathy in COPD patients. The steroid-induced quadriceps myopathy was characterized by increased variation in diameter of fibres, with several angular atrophic fibres, diffuse necrotic and basophilic fibres. Between the fibres, connective tissue was increased. These patients had severe peripheral muscle weakness and lower quadriceps force than the control COPD patients. Hence, large doses of corticosteroid or long-term steroid therapy could result in skeletal muscle impairment, limiting exercise capacity in patients.

Azathioprine (AZA). Until recently, it was generally accepted that AZA had no side effects on skeletal muscle. Simon et al (1997), however, showed that 1 μmol L^{-1} AZA *in vitro*

inhibited the respiratory control ratio by -10.5% in rat kidney mitochondria. There is no evidence to demonstrate exercise limitation caused by AZA.

Cyclosporine A. CyA is the most effective immunosuppressant contributing to the survival rate of all organ transplantation recipients. CyA, however, has significant side effects after HLTx and LTx. The major adverse effects are systemic hypertension and nephrotoxicity.

Cyclosporine A-Induced Systemic Hypertension and Nephrotoxicity. Hosenpud et al (1997) concluded that the prevalence of systemic hypertension and of renal dysfunction in LTx was 44.2% and 7.9% in the first year and 56.9% and 9.4%, in second years after operation, respectively. Studies in both animals (L'Azou et al 1994; Takeda et al 1995) and humans (Myers et al 1988; Pei et al 1995) have been documented that CyA is a major toxic agent contributing to these complications. CyA can cause small artery general constriction in organs and peripheral tissue (Bartholomeusz et al 1996). It may be the primary pathological change in CyA-induced nephrotoxicity and systemic hypertension. There are numerous of explanations for CyA-induced vasoconstriction. Firstly, CyA may cause an increase in the synthesis and release of endothelin or an increase in endothelin type A receptors of the arteries (Bartholomeusz et al 1996; Takeda et al 1995). The endothelin is a potent vasoconstrictor of the peripheral vascular system. Secondly, CyA increases the Ca^{2+} influx to vasoconstrictor hormones in smooth muscle cells, which likely increases vasoconstriction (Lo-Russo, et al 1996). Thirdly, CyA can impair mitochondrial function (Jung and Reinholdt 1987) which may over-produce oxidants leading to renal and systemic vasoconstriction and resulting in nephrotoxicity and systemic hypertension (Wolf et al 1994; Assis et al 1997; Baliga et al 1997; Krysztopik et al 1997).

Studies for hypertension have revealed histological and biochemical alterations in skeletal muscle of these patients. Hypertensive individuals have been found to exhibit a lower (35% *vs.* 40%, $P < 0.05$) proportion of type I fibres and a higher proportion of type II fibres (Juhlin-Dannfelt et al 1979). Ben Bachir-Lamrini et al (1990) demonstrated that a group of

spontaneously hypertensive rats (SHR) displayed a significantly decreased proportion of slow-twitch fibres in slow muscle (soleus) from 4 weeks age compared with normotensive rats. They suggested that muscle fibre transition may be caused by an increased level of plasma catecholamines in SHR. In terms of muscle metabolism, both muscle biopsy and NMR studies demonstrated an ~30% decrease in PCr content in resting muscle and slower PCr regeneration during post exercise recovery in a group of hypertensive patients (Ronquist et al 1995). Hypertensive patients have an impaired exercise tolerance which is ~30% less than age matched controls (Lim et al 1996). Rieder et al (1996) demonstrated that hypertension reduced skeletal muscle force generation, decreased muscle performance and increased muscle fatigue in renal hypertensive rats. Consequently, CyA induced hypertension may aggravate the muscle deficiencies and reduce exercise tolerance.

CyA induced myopathy. CyA induced myopathy is rare, but there have been some cases reported (Noppen 1987; Goy et al 1989; Fernandez-Sola et al 1990; Arellano and Krupp 1991). The characteristics of this myopathy include myalgia, weakness, muscle cramps and rhabdomyolysis in clinical manifestations and type II fibre atrophy, segmentary necrosis, Z-line streaming, disruption of myofibrils and abnormal mitochondria. CyA-induced myopathy may also be related to other drugs and agents used after cardiac transplantation, such as, HMGRIs, the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor and the newer antilipemic drugs (Corpier et al 1988; East et al 1988; Smith et al 1991; Alejandro and Petersen. 1994). CyA hypomagnesaemia, which may cause skeletal muscle mitochondrial dysfunction and myopathy (Larner et al 1994).

CyA and Mitochondrial function. Jung and Pergande (1985) and Zenatti et al (1988) demonstrated that CyA can inhibit mitochondrial respiration in the isolated mitochondria of rats. Jung and Reinholdt (1987) have also shown that CyA depressed mitochondrial function of human kidneys in a dose dependent manner. In addition, CyA can significantly decrease mitochondrial function in the liver (Samuta et al 1993) and cardiac myocytes (Altschuld et al

1992). The effect of CyA on mitochondrial O_2 consumption in skeletal muscle has been demonstrated both *in vivo* and *in vitro*. Hokanson et al (1995) examined O_2 consumption in isolated mitochondria from rat skeletal muscle. The results showed that addition of CyA (25 $\mu g\ ml^{-1}$) into the test chamber reduced O_2 consumption significantly. Mercier et al (1995) found a reduction in the O_2 consumption of skeletal muscle in isolated mitochondria of rats that had been fed a high dosage of CyA (20 mg kg^{-1} day for14 days). The study also showed a significant linear correlation between endurance exercise time and State 3 respiration of mitochondria.

Previous studies have shown that oxidants, including free radicals and lipid peroxidation (Wolf et al 1994, 1997), could be induced by CyA. It has been documented that oxidants damage membrane lipids (Ames et al 1995), protein (Sohal and Dubey 1994), electron chain components and ATPase (Zhang et al 1990), and DNA metabolism (Fraga 1990). There were reports, however, that CyA may act as a mitochondrial permeability transition pore (MPTP) blocker to reduce the ischemia-reperfusion damage in rat heart mitochondria (Griffiths and Halestrap 1993) and prevented the mitochondrial injury by anoxia-reoxygenation in rat liver mitochondria (Tanaka et al 1998). CyA blocks mitochondrial Ca^{2+} release resulting in Ca^{2+} storage increased in mitochondria (Crompton et al 1988, Richter et al 1990). The consequences of Ca^{2+} overload in mitochondria have been discussed in Section 2.3.6.2.

2.4.4. Summary

During the past 15 years HLTx and LTx have become established modes of treatment for many forms of end-stage heart-lung and pulmonary disease. Post LTx, the survival rate and quality of life of recipients have shown dramatic improvement followed by improved cardiac and pulmonary function. The exercise capacity for these organ transplant recipients, however, is still lower than the predicted levels. The exercise limitation is characterised by poor exercise tolerance, lower $\dot{V}O_2$ peak and an early rise in blood lactate. Potential causes may be

related to preoperative cardiopulmonary disease and long-term reduction of physical activity which lead to skeletal muscle deconditioning, either physical, pathological or both. Exercise limitation may also be affected by several post-transplant factors. Muscle fibre atrophy, a lower proportion of type I fibre, and lower aerobic metabolic capacity may limit exercise tolerance. Immunosuppressive medications can also impair skeletal muscle, especially CyA and corticosteroids. CyA not only causes renal failure and system hypertension but may also impair mitochondrial function in skeletal muscle in lung transplant patients.

CHAPTER 3. GENERAL METHODS

3.1 OVERVIEW

This thesis comprises three studies as follows:

- Study I. Mitochondrial capacity and metabolism in skeletal muscle post lung transplantation (chapter 4).

The first study investigates mitochondrial ATP production rate (MAPR), metabolic enzyme activity, muscle fibre type and resting muscle metabolites in a group of LTx recipients and age and sex-matched controls. The study also examined the response to incremental exercise in these groups.

- Study II. The effects of cyclosporine on *in vitro* MAPR in rat skeletal muscle (Chapter 5).

The second study used a rat model to examine whether MAPR in skeletal muscle is suppressed by cyclosporine A at 50 $\mu g\ ml^{-1}$, 25 $\mu g\ ml^{-1}$ and 1 $\mu g\ ml^{-1}$ concentrations. This part of the study provided further evidence of the CyA toxicity on skeletal muscle mitochondrial function and may help to understand the cause of exercise limitation in patients post lung transplantation (Chapter 4).

- Study III. Mitochondrial capacity and exercise metabolism in the skeletal muscle of resistance-trained individuals (Chapter 6).

The third study examines the skeletal mitochondrial capacity in resistance-trained subjects and untrained controls including the proportion of muscle fibre types, metabolic enzyme activities, metabolites in resting and fatigued muscles during intense fatiguing exercise and MAPR with various substrates. Exercise capacity and function of quadriceps skeletal muscle were also tested in the third study.

A total of 30 human subjects participated in Study I and III and they performed a variety of

exercise tests. These tests included assessment of $\dot{V}O_2$ peak (Studies I and III), and tests to assess muscle strength, power and fatigability (Study III). Arterialised venous blood samples were obtained from a superficial dorsal vein of a hand at rest and during the exercise tests in Studies I and III. Muscle samples were obtained from the vastus lateralis at rest in Study I as well as at rest and immediately after exercise to fatigue in Study III. For Study II, red gastrocnemius muscle samples were obtained from rats after euthanasia.

Blood samples were analysed for plasma lactate and blood gases (Studies I and III). Muscle samples were analysed for MAPR (Studies I, II and III), fibre type composition (Studies I and III), metabolic enzyme activities, resting muscle metabolites (Studies I and III) and metabolites in both resting and fatigued muscle (Study III).

Full details of exercise tests and subjects (or animals) are given in the relevant chapters. This chapter describes the measurement of maximal aerobic power and the general methods employed to analyse blood and muscle samples.

In both Study I and III, subjects were required to complete a detailed medical questionnaire and gave written informed consent (Appendix I) before commencing the study. The Human Research Ethics Committee of Victoria University of Technology approved all protocols and procedures conducted at Victoria University. The Hospitals Ethics Committee of the Alfred Group approved all protocols and procedures conducted at the Alfred Hospital. The Animal Experimentation Ethics Committee, Victoria University of Technology, approved all procedures in Study II.

3.2. LUNG FUNCTION TESTING

All subjects performed spirometry to determine their vital capacity (VC) and forced-expired volume in one second, (FEV_1). Healthy subjects were tested on a spirometer (Minato Osaka,

Japan) whilst transplant patients were tested at the Alfred Hospital on a Jaeger Masterlab 3.30 spirometer (Wuerzburg, West Germany).

3.3. MEASUREMENT OF MAXIMAL AEROBIC POWER

The subjects refrained from exercise, alcohol and caffeine consumption for 24 hours prior to the test. On the testing day, subjects had a light breakfast. When they arrived at the laboratory, they were weighed and a catheter was inserted into a superficial vein in the dorsum of the right hand for arterialised venous blood sampling (see 3.4.1). Maximal aerobic power was determined using an incremental exercise test on an electrically braked cycle ergometer. This measurement included $\dot{V}O_2$ peak, peak carbon dioxide output ($\dot{V}CO_2$peak), minute ventilation at peak exercise ($\dot{V}$Epeak), peak workrate, maximal exercise duration and HRpeak. The protocol details of measurements see the relevant Chapter. During the incremental exercise test, blood samples were obtained for determination of blood gas and plasma lactate (see blow).

3.4. BLOOD ANALYSES

3.4.1. Blood Sampling

Prior to commencement of the exercise test, a 20 G indwelling catheter was inserted into a superficial vein in the dorsum of the right hand. The catheter was attached to an extending line, with a three-way tap allowing multiple samples to be taken. A plastic cover (Tegaderm ™) and a common surgical glove protected the hand with a venous catheter. The hand was placed in a hot water bath at 45 °C for 10 minutes to achieve arterialisation of venous blood.

Arterialised venous blood samples were drawn at rest, every minute during the increment exercise test to voluntary exhaustion. Two syringes of blood were drawn at each sampling time, the first one for plasma gases and the second for lactate measurements. Heparinised saline was flushed (1-2 ml) intermittently to maintain patency. Arterialisation of the venous blood was maintained by persistent immersion of the hand in the hot water bath throughout exercise (~45 °C). Regular blood gas analysis was used to confirm successful arterialisation indicated by oxygen saturation greater than 90%.

3.4.2. Blood Processing

The blood gas syringe was capped tightly and placed on ice for plasma oxygen partial pressure (PO_2), and partial pressure of carbon dioxide (PCO_2) analyses (865 Ciba Corning, Medfield, MA, USA) for healthy subjects. The blood gas samples in the LTx patients in Study I used an automated blood gas analyses (Radiometer, Copenhagen, Denmark). While blood samples from the second syringe were centrifuged and 250 µl of aliquot of plasma was deproteinised in 500 µl of 3.0 mmol L^{-1} perchloric acid. After centrifugation the supernatant was drawn off and stored in a freezer at –75 °C for later lactate analysis. Plasma lactate was determined in triplicate, using an enzymatic technique with spectrophotometric detection according to the method of Lowry and Passonneau (1972). The lactate threshold was assessed from a plot of plasma lactate concentration against workrate and $\dot{V}O_2$ using the log-log transformation model that was described by Beaver et al. (1985).

3.5 MUSCLE SAMPLES AND ANALYSES

3.5.1 Vastus Lateralis Muscle Needle Biopsies

Muscle biopsy samples were obtained from the vastus lateralis muscles using the needle

biopsy technique as described by Bergstrom (1962). Briefly, with the subject resting supine, the skin was prepared with iodine solution (Betadine). Lignocaine (2%) local anaesthetic was infiltrated into the skin, subcutaneous tissue and fascia. Three small incisions for Study III and two incisions for Study I were made into the skin. A 5-mm muscle biopsy needle was used to obtain 70-100 mg of skeletal muscle. One portion of muscle (15-20 mg), for fibre type analysis, was embedded in the mounting medium, immediately immersed in isopentane cooled in liquid nitrogen (LN_2) and subsequently stored in LN_2. Two pieces of muscle (15 mg and 20 mg) were immediately frozen in liquid nitrogen and stored in liquid nitrogen for enzyme activity and metabolite analyses. The remaining piece of fresh muscle (25-40 mg) was placed on ice and used for the determination of MAPR.

3.5.2. Muscle Analyses

All muscle analyses were conducted in the Exercise Metabolism Unit Laboratory, School of Life Sciences and Technology, Victory University of Technology.

3.5.2.1 Muscle Fibre Types

Muscle fibre types were determined using the myofibrillar ATPase method as described by Dubowitz and Brookes (1985). The muscle cross-section about 10 μm thick was cut on a cryostat at –20 °C (Microm GMBH D-6900 500, Heidelberg, Germany). The sections were preincubated at pH 10.35, 4.6 and 4.3, and after staining for myosin ATPase, were visually counted to determine the percentage of each type. Fibres were classified into type I (slow-twitch) and types IIa and IIb (fast-twitch) according to myofibrillar ATPase staining patterns.

3.5.2.2. Muscle Metabolite Analyses

Muscle freeze-drying and extraction

Previously stored muscle samples (15–20 mg) for analysis of metabolites were weighed at

minus 20 °C and freeze-dried (Edwards, Modulyo) for 48 hours, re-weighed and powdered. Approximately 1mg of dry muscle powder was apportioned for glycogen determination. Portions of approximately 2 mg of dry muscle powder were used for the analysis muscle of metabolites.

The samples for glycogen determination (~1 mg dry muscle powder) were hydrolysed in 250 µl aliquot of 2 mmol L^{-1} hydrochloric acid (HCL) at 100 °C for 2 hours. After hydrolysis with periodic mixing, the samples were neutralized by the addition of 0.75 ml of 0.667 mmol L^{-1} sodium hydroxide (NaOH), and then stored at –80 °C to be analysed.

The samples for muscle metabolite analyses (~2mg of dry muscle powder) were extracted in a 5 step procedure according to the method of Harris et al (1974). (1). A 250 µl aliquot of 0.5 mmol L^{-1} perchloric acid (PCA) with 1 mmol L^{-1} methylenediaminetetra-acetic acid (EDTA) was added to the tubes containing the muscle powder, then mixed on a vortex for 10 minutes. The tubes containing muscle samples were placed on ice. PCA-EDTA solution was pre-cooled on ice. (2). After mixing, the samples were centrifuged (Heraeus Sepatech Biofuge 28RS, USA) at 28,000 RPM (51,000 G) at 0°C for two minutes. (3). A 200 µl aliquot supernatant was transferred to a pre-cooled and appropriately labeled eppendorf tube. The supernatant was neutralized with 50 µl aliquot of 2.1 mmol L^{-1} ice-cold $KHCO_3$, then mixed and put on ice for 5 minutes to complete precipitation of perchlorate ions. (4). Again, the samples were mixed and spun on a centrifuge as before. (5) Finally, the supernatant was transferred to a pre-cooled, labeled cryule then stored at –80 °C until analysis.

Analyses of Metabolites

The PCA extract was used to determine metabolites. Muscle adenine nucleotides, ATP, ADP, AMP and their degradation product IMP, were analysed in a single measurement by a reverse phase high performance liquid chromatography (HPLC) (ICI Instruments, Australia) using the method of Wynants and VanBell (1985). ATP, PCr and lactate were determined enzymatically in triplicate, and Cr in dulplicate, using fluorometric detection (Turner Fluorometer Model

112. California USA) according to the methods of Lowry and Passoneau (1972). The hydrolysis of glucose derived from glycogen was analysed in duplicate according to the procedure of Lowry and Passonneau (1972).

3.5.2.3. Enzyme Analyses

Metabolic enzymes measured were the TCA enzymes CS and KGDH, the fatty acid β-oxidative enzyme HAD, the glycogenolytic enzyme PHOSPH and the glycolytic enzymes PFK, PK and HK. The muscle samples for analysis of PHOSPH, PFK, PK, HK and HAD were homogenized with homogenising solution containing 0.17 mmol L^{-1} KH_2PO_4, 0.02% BSA and 5 mmol L^{-1} mercaptoethanol and pH 7.4 (1:100 wt/vol) for 1 minute at maximal speed using an electric homogeniser (OMNI International S/N TH-1276, Warrenton, USA). The activity of enzymes was determined on tissue homogenate using fluorometric methods of Green et al. (1984). The same homogenising solution was used for KGDH. The reaction medium for analysis of KGDH as described by Read et al. (1977) and analysis used modified fluorometric method. The muscle samples for assays of CS were homogenised with homogenising solution containing Triton X-100 (0.05% vol/vol), 50 mmol L^{-1} KH_2PO_4 and 1 mmol L^{-1} EDTA, pH 7.5 (1:100 wt/vol) for one minute at maximal speed using an electric homogeniser as above. The activity of CS was determined using the spectrophotometric method as described by Srere (1969). Activity of enzyme is expressed by muscle weight (mmol min^{-1} kg^{-1} wet weight of muscle) and is also expressed by muscle protein (mmol min^{-1} g^{-1} protein of muscle). The protein content in the muscle sample was determined from muscle sample for enzyme activity measurement according to the method of Lowry et al. (1951). All enzyme activity measurement was made duplicate at 25 °C temperature.

3.5.2.4. Measurement of Mitochondrial ATP Production Rate (MAPR)

Mitochondrial Preparation

Homogenising solutions for the preparation of isolated mitochondria and the isolation procedures are described by Wibom et al. (1990). Fresh muscle was placed on a petri-dish over ice, dissected free of fat and connective tissue, cut into small pieces and weighed. The muscle sample was put into 1 ml of pre-cooled homogenising solution (solution A) then twice homogenised for 5 seconds using an electric homogeniser (OMNI International S/N TH-1276, Warrenton, CA. USA). Solution A contained tris (hydroxymethyl) amino methan (50 mmol L^{-1}), KCl (100 mmol L^{-1}), $MgCl_2$ (5 mmol L^{-1}), ATP (1.8 mmol L^{-1}), EDTA (1 mmol L^{-1}), pH 7.2 with HCl. Homogenisation was performed at the lowest operating speed of the instrument. The homogenate was centrifuged for 3 minutes at 650 g and the supernatant was centrifuged at 15,000 g for 3 minutes. The pellet was then re-suspended in 1ml of solution A and again centrifuged at 15,000 g for 3 minutes.

The pellet was suspended in 200 µl of solution B. The solution B contained sucrose (180 mmol L^{-1}), KH_2PO_4 (35 mmol L^{-1}), Mg acetate (5 mmol L^{-1}), EDTA (1 mmol L-1) and pH 7.5 with KOH. A 100 µl of aliquot of this mitochondrial suspension was diluted with 400 µl of solution B (1:5 diluted mitochondrial suspension). Next 10 µl of the 1:5 diluted mitochondrial suspension was further diluted with 490 µl (1:250 diluted mitochondrial suspension) of the ATP-monitoring reagent (AMR) solution. AMR was prepared by diluting the commercially provided solution (FL-MXB, Sigma) 12.5-fold with solution C. Solution C contained inorganic pyrophosphate (0.05 mmol L^{-1}), Mg acetate (0.5 mmol L^{-1}), BSA (1 mg ml^{-1}), sucrose (180 mmol L^{-1}), KH_2PO_4 (35 mmol L^{-1}), EDTA (1 mmol L^{-1}), pH 7.5 with KOH. Therefore, the final mitochondrial suspension used for MAPR was a 1:250 dilution of the origenial suspension. This suspension was placed on ice ready for MAPR analysis. The remaining 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). The

remaining 100 µl of the undiluted mitochondrial suspension was used for determination of GDH. The steps of mitochondrial preparation are shown in the Figure 3.1.

Measurement of MAPR

MAPR was determined at 25 °C, using the method and substrate concentrations described by Wibom and Hultman (1990). Aliquots of mitochondrial suspension were added to cuvettes containing purified ADP, AMR (FL-MXB, Sigma) and a variety of substrates. The ADP (Boehringer mannheim, Germany) was purified using the ion-exchange chromatography method as described by Lundin (1978). The substrate combinations utilised in the present study were as follows: 1.0 mmol L^{-1} pyruvate + 1.0 mmol L^{-1} malate (P + M), 0.005 mmol L^{-1} palmitoyl-L-carnitine + 1.0 mmol L^{-1} malate (PC + M), 20 mmol L^{-1} succinate + 0.1 mmol L^{-1} rotenone (S + R), 10.0 mmol L^{-1} α-ketoglutarate (α-KG) and 1.0 mmol L^{-1} pyruvate + 0.005 mmol L^{-1} palmitoyl-L-carnitine + 10 mmol L^{-1} α-ketoglutarate + 1.0 mmol L^{-1} malate (PPKM). A blank was prepared containing purified ADP (30 µmol L^{-1}) and mitochondrial suspension (10 µl of 1:250 diluted mitochondrial suspension solution in human studies, see Figure 3.1), but no substrates, in which ATP production may be due to adenylate kinase reaction and other nonspecific reactions (Wibom et al. 1990). The final volume in the cuvette for MAPR was 1 ml. MAPR was determined by the rate of light production, which was measured on a custom designed luminometer comprising a photomultiplier tube attached to a luminescence spectrometer (Aminco Bowman AB2, Urbana, USA). Cuvettes containing the various substrates in the five combinations mentioned above and a blank with 10 µl of 1: 250 diluted mitochondrial suspension were incubated in a 25 °C water bath for 5 minutes after adding ADP. After incubation, light emission in the cuvettes was measured in the luminescence spectrometer chamber (25 °C). During 30 seconds between measurement, the cuvettes were incubated in a 25 °C water bath. At the end of the MAPR assay, 10 µl of a 190 nmol L^{-1}ATP standard (FL-AAS, Sigma) was added to the test cuvette as an internal ATP standard, to allow for calculation of MAPR. Rates are expressed as mmol min^{-1} g^{-1}

mitochondrial protein and mmol min^{-1} kg^{-1} muscle, referring to the protein content in the mitochondrial suspension and the muscle wet weight, respectively. All measurements were made in duplicate and were completed within 4 hours after biopsy. Mitochondrial suspension protein was measured in triplicate used the method described by Lowry et al. (1951).

3.5.2.5. Measurement of Glutamate Dehydrogenase

GDH, a glutamate oxidative deamination enzyme, is specific as a mitochondrial marker enzyme and was used to determine mitochondrial yield in the suspensions. Mitochondrial GDH activity was determined on the mitochondrial suspension before and after disruption of the mitochondrial membrane with Triton X-100. A 30 µl aliquot of the original mitochondrial suspension (see Figure 3.1) was diluted with 170 µl of solution containing 50 mmol L^{-1} KH_2PO_4 and 1 mmol L^{-1} EDTA, pH 7.5 for the measurement of GDH activity in the mitochondrial suspension prior to rupture of the mitochondrial membrane (GDHmb). For the measurement of GDH activity in the mitochondrial suspension after rupture of the mitochondrial membrane (GDHma) assay, a 15 µl of same mitochondrial suspension was diluted using 185 µl of the solution containing Triton X-100 (0.05% vol/vol), 50 mmol L^{-1} KH_2PO_4 and 1 mmol L^{-1} EDTA, pH 7.5. From these measurements, the activity of GDH in intact mitochondria (GDHim = GDHma-GDHmb) can be determined. The total GDH (GDHt) activity was measured on another piece of muscle. This muscle sample was homogenised for 1minute at maximal speed of the electric homogeniser (OMNI International S/N TH-1276, USA). The homogenising solution employed was the same as that used in the determination GDHma. GDH activity in all homogenates was determined at 35 °C, according to the method of Schmidt (1974) and modified for fluorometric detection (Aminco Bowman AB2, Urbana, USA). GDHt values are shown in Appendix III. The ratio of GDHim and GDHt can be used for the calculation of MAPR per unit muscle mass and mitochondrial yield according to the method of Wibom and Hultman (1990). Details of this method are shown in Appendix III.

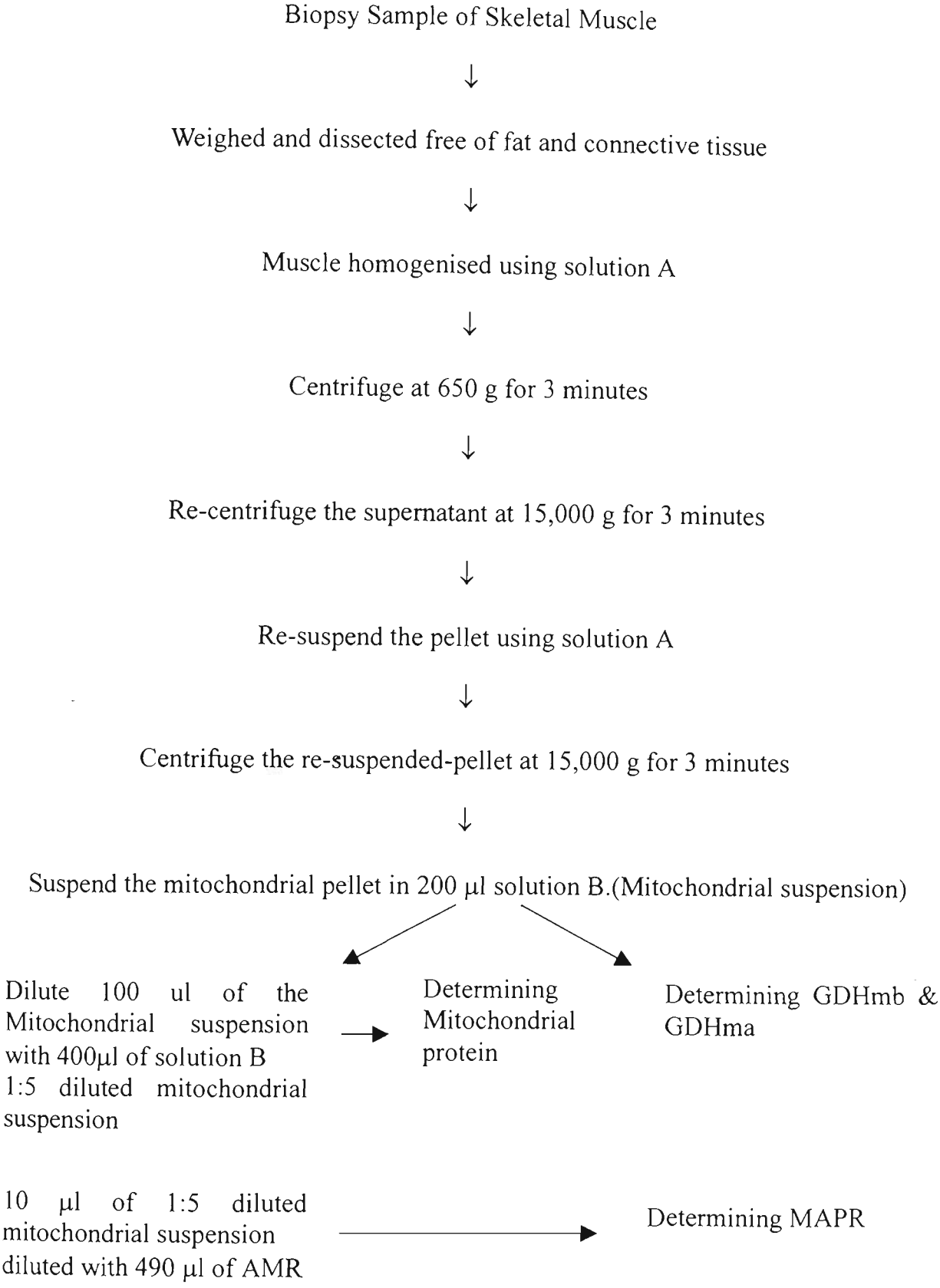


Figure 3.1. Methods of Mitochondrial Preparation

AMR, the ATP-monitoring reagent solution; GDHmb, GDH activity before disrupture mitochondrial membrane; GDHma, GDH activity before disrupture mitochondrial membrane.

3.6. CYCLOSPORINE A STUDY

Eight female Sprague-Dawley rats were used in this study. The details of the methods in this study are described in Chapter 5.

3.7. STATISTICAL ANALYSES

The statistical analyses are described separately in each chapter.

CHAPTER 4. MITOCHONDRIAL CAPACITY AND METABOLISM IN SKELETAL MUSCLE POST LUNG TRANSPLANTATION

4.1. INTRODUCTION

Lung transplantation (LTx) has become an established mode of treatment for many end-stage pulmonary diseases. Procedure presently in the LTx have recently been reviewed (Williams and Snell 1997). Lung transplantation includes heart-lung transplantation (HLTx), double or bilateral lung transplantation (DLTx or BLTx) and single lung transplantation (SLTx). DLTx is either performed as bilateral sequential lung transplantation or infrequently en-block double lung with bronchial revascularisation. Recently, live donor bilateral lobar transplantation has been reported (Starnes et al 1994). In the period 3 to 6 months after operation, HLTx and DLTx recipients typically have near normal spirometry (Madden et al 1992; Bando et al 1995) with mildly impaired diffusion capacity (Williams et al 1990). SLTx typically results in persistent abnormal spirometry, which may be caused by the reduced diffusion capacity in the remaining native lung (Grossman et al 1990, Williams et al 1990, Maurer et al 1991, Levy et al 1993).

After the first several months recovery, HLTx, DLTx and SLTx recipients are remarkably similar in the terms of the peak oxygen uptake, with values of ($\dot{V}O_2$ peak during exercise 41% to 57% of the predicted levels (Miyoshi et al 1990; Theodore et al 1992; Williams et al 1992a; 1992b; Levy et al 1993). During incremental exercise testing these patients have a low peak workrate and an early rise in blood or plasma lactate (Madden et al 1992; Levy et al 1993; Orens et al 1995). A number of potential causes for exercise limitation in LTx patients have been considered. These include poor motivation, reduced cardiac output, low

haemoglobin (Hb) levels, and peripheral skeletal muscle defects resulting in reduced uptake/utilisation of delivered oxygen (Miyoshi et al 1990; Theodore et al 1987; Howard et al 1994).

The defects in skeletal muscle may be the result of a pre-transplant skeletal muscle injury, perhaps due to physical deconditioning or muscle disuse. Peripheral muscle deconditioning caused by immobilisation in human is characterised by a reduced muscle strength (Imms et al 1977) and reduced muscle function as indicated by isoknetic peak torque (Halkjœr-Kristensen and Ingemann-Hansen 1985c). Deconditioned skeletal muscle exhibits muscle fibre atrophy (Halkjœr-Kristensen and Ingemann-Hansen 1985a; Bloomfield 1997) and is associated with a low (from 42% decreased to 37%; $P < 0.001$) proportion of type I fibres (Halkjœr-Kristensen and Ingemann-Hansen 1985a). The activity of muscle oxidative enzymes is also low in the subjects with muscle immobilisation (Häggmark et al 1981; Halkjœr-Kristensen and Ingemann-Hansen 1985b; Jansson et al 1988; Blakemore et al 1996). Similar skeletal muscle fibre type changes have been reported in cardiopulmonary diseases, such as chronic obstructive pulmonary diseases (COPD) (Jakobsson et al 1990, Whittom et al 1998), chronic pulmonary emphysema (Sato et al 1997) and congestive heart failure (CHF) (Sullivan et al 1990). Reduction in the oxidative enzyme activity and abnormalities of metabolism in skeletal muscle have also been reported in CHF (Chati et al 1994), COPD (Jakobsson et al 1995; Maltais et al 1996; Pouw et al 1998) and cystic fibrosis (CF) patients (De Meer et al 1995). Post transplant, the potential factors resulting in skeletal muscle defects include increased protein catabolism, particularly in response to sepsis (Hasselgren et al 1989; Hasselgren and Fischer 1997), muscle persistence in deconditioning and the effects of medications, especially corticosteroids and CyA (see Section 2.4.3.2). Evans et al (1997) demonstrated a low pH in skeletal muscle at rest and an earlier decline in muscle pH during submaximal exercise in LTx patients. This study indicates the oxidative capacity and/or mitochondrial capacity in skeletal muscle is impaired in LTx recipients. The authors speculated that the reduced oxidative

capacity of peripheral skeletal muscle may have been the cause of exercise limitation.

Accordingly, the hypothesis of this study is that LTx patients have a reduced oxidative capacity in peripheral skeletal muscle. Hence, this study investigates oxidative capacity in the skeletal muscle of LTx patients through an examination of measurement of fibre-type composition, resting muscle metabolites, muscle metabolic enzyme activities and the measurement of mitochondrial ATP production rate. An incremental exercise test is also employed in this study to determine endurance exercise performance indicated by the duration of exercise and $\dot{V}O_2$ peak.

4.2. METHODS

4.2.1. Subjects

Seven LTx recipients, 4 women and 3 men, ranging in age from 25 to 53 years, 3 to 24 months post transplant, and 7 age (sex)-matched control subjects (MC) volunteered to participate in this study. Subject characteristics and spirometry are listed in Table 4.1. The transplant recipients had stable lung function and showed no evidence of recent infection or rejection, as they had fully rehabilitated following lung transplant procedures. The respective diagnoses and operative procedures are shown in Table 4.2. Determination of vital capacity (VC) and forced-expired volume in one second (FEV_1) were described in Section 3.2. Predicted values of VC and FEV_1 were calculated according to the equations in Crapo et al (1981) which are listed in Appendix II. The predicted value of pulmonary diffusing capacity for carbon monoxide (TLCO) was calculated in LTx patients only using equations from Crapo & Morris (1981) which are also listed in Appendix II.

All the LTx recipients received immunosuppressive medications including cyclosporine A (CyA) and prednisolone (PNL). Six of the seven patients also received azathioprine (AZA)

(Table 4.2). Other major medications included calcium blockers, angiotension converting enzyme (ACE) inhibitors, antiviral antibiotics and calcium, potassium and magnesium supplements (Appendix II). The controls were sedentary and did not exercise regularly. The study of transplantation patients involved a collaboration with the Department of Respiratory Medicine, and the Heart and Lung Transplantation Medical Team of the Alfred Healthcare Group. The Victoria University of Technology Human Research Ethics Committee and the Alfred Group of Hospitals Ethics Committee approved all protocols and procedures. Written informed consent was obtained from all participants.

Table 4.1. Characteristics of LTx Patients and MC Subjects

	LTx	MC
Female/Male	4/3	4/3
Age (year)	37 ± 4	37 ± 4
Height (cm)	170 ± 3	172 ± 4
Weight (kg)	66.4 ± 3.9	69.4 ± 4.4
FEV_1 (L)	2.64 ± 0.3*	3.87 ± 0.2
% predicted	75 ± 9†	109 ± 7
VC (L)	3.46 ± 0.3*	4.78 ± 0.2
% predicted	79 ± 7†	112 ± 8
FEV_1/VC (%)	77.2 ± 6.8	81.1 ± 2.0
% predicted	96 ± 8	98 ± 3
TLCO (ml min^{-1}mmHg)	18.0 ± 2	N/A
% predicted	62 ± 5‡	-

Values are mean ± SEM. n = 7. FEV_1, forced expiratory volume in one second; VC, vital capacity; TLCO, diffusing capacity for carbon monoxide; LTx, lung transplantation; MC; age (sex)-matched controls. *$P < 0.05$. Values compared with controls. †$P < 0.05$, and ‡$P < 0.01$. Values compared with predicted values.

Table 4.2. Clinical Characteristics of LTx Patients

LTx	Diagnosis	Operation	P-O	Blood Pressure	Haemoglobin	Immunosuppressive Medication			
			months	Dia/Sys (mmHg)	(g L^{-1})	PNL (mg/day)	CyA po (mg/day)	[CyA] (ng ml^{-1})	AZA (mg/day)
1	IPF	SLTx	15	150/80	117	15	350	224	100
2	PL	SLTx	9	140/80	106	15	300	336	NIL
3	PPH	HLTx	24	140/90	126	10	300	412	25
4	PH	DLTx	8	150/90	116	15	400	251	100
5	OB	DLTx	21	130/70	115	15	500	198	100
6	CF+ A_1TD	DLTx	3	155/80	126	20	300	222	75
7	CF	DLTx	4	120/70	113	15	450	221	100

Definition of abbreviations: P-O, post operation; Dia/Sys, diastolic/Ststolic blood pressure; LTx, lung transplantation; SLTx, single lung transplantation, HLTx, heart-lung transplantation; DLTx, double lung transplantation; IPF, idiopathic pulmonary fibrosis; PH, pulmonary hypertension; PL, pulmonary lymphangiolieomyomatosis; PPH, primary pulmonary hypertension; OB, obliterans bronchiolitis; CF, cystic fibrosis; A_1TD, α_1-antitrypsion deficiency; PNL, prednisolone; CyA, cyclosporine A; [CyA], blood cyclosporine A concentration; AZA, azathioprine; po, per os (by mouth).

4.2.2. Incremental Exercise Tests

At least 2 hours after a light breakfast, each subject performed an incremental exercise test on an electronically braked cycle ergometer. The LTx patients performed this test on a Sensormedics 2900 system (Sensormedics Co., Yorba Linda, USA) in the Respiratory Medicine Laboratory of the Alfred Hospital. The controls were tested on a system that included a Lode N.V. cycle ergometer (Groningen, Netherlands). After 5 minutes resting on the cycle ergometer, subjects cycled at 60-80 revolutions per minute (rpm). Workrate for controls was increased by 25 watts each minute until volitional exhaustion. Volitional exhaustion was defined as the inability to maintain pedal cadence of 50 rpm. During the test expired gases were directed into a mixing chamber through a Hans-Rudolph 2-way non-rebreathing valve. The expired volume was measured using a flow transducer (KL Engineering, Sunnydale, California, USA) and mixed expired oxygen and carbon dioxide were analysed by rapidly responding gas analysers (Amtek S-3A, Pittsburg, USA). The composition of the expired air was calculated over 15 seconds using gas analysers (TurboFit, California, USA.). The flow transducer was calibrated, both before and after each test, using a 3-litre syringe. The gas analysers were calibrated immediately prior to each test, and rechecked after the test, using commercially prepared gas mixtures of known composition. Heart rate and rhythm were monitored by an electrocardiogram (ECG) (Mortara, X-Scibe, Milwaukee WI, USA). These tests measured $\dot{V}O_2$ peak, $\dot{V}CO_2$peak, peak workrate (workrate-peak), maximal exercise duration and HRpeak. Predicted values of $\dot{V}O_2$ peak and HRpeak were calculated using the equations described by Jones et al (1985) and Spiro (1977), respectively, which are listed in Appendix II. Maximum voluntary ventilation (MVV) was calculated from following equation: $MVV = FEV_1 \times 35$ (Gandevia and Hugh-Jones 1957).

The protocol of the incremental exercise tests for LTx patients was the same as the controls, but workrate was increased by 16.3 watts per minute. The ventilatory function of the LTx

patients and the controls was determined on the day of the incremental exercise test.

The two systems, which were used to determine the incremental exercise tests for LTx in Alfred hospital and for MC in VUT, were compared in 3 untrained subjects. Linear regressions of average $\dot{V}O_2$ peak, $\dot{V}CO_2$peak and $\dot{V}$Epeak values were used to compared the two systems. There were no statistical differences between the two systems ($P > 0.05$; see Appendix V).

4.2.3. Blood Sampling

The methods of catheterisation and arterialisation of the venous blood for blood sampling are described in Section 3.4.1.

4.2.4. Muscle Biopsy and Muscle Analysis

Within 2 to 7 days after the incremental exercise test a resting muscle biopsy was obtained from the vastus lateralis muscle. In the transplant group this procedure was performed whilst the patients were sedated with pethidine and midazolam for a routine bronchoscopy. With the subject resting supine, 2% lignocaine local anaesthetic was infiltrated into the skin, subcutaneous tissue and fascia overlying the belly of the vastus lateralis. Two small incisions (~5 mm) were made on the leg of each subject for resting muscle biopsies. Procedure, technique and muscle samples management are described in Section 3.5.1.

The muscle samples from LTx patients and controls were analysed for fibre types, metabolites, metabolic enzymes and MAPR. The details of methods have described in Section 3.5.2.

4.2.5. Statistical Analysis

Data is expressed as mean ± SEM. Data from patients and control subjects were compared for independent variables using two-tailed Student's *t*- test using Microsoft Excel software package. *P* value < 0.05 was considered to indicate a significant difference. Linear regressions were determined from the pooled data of the two groups and calculated using Microsoft Excel software package.

4.3. RESULTS

4.3.1. Exercise Performance and Pulmonary Function

Based on predicted values, lung function tests revealed mild mixed obstructive and restrictive ventilation defects in LTx and normal spirometry in controls (Table 4.1). Despite a lower workrate increment in the exercise tests, the duration of exercise in LTx was significantly shorter ($P < 0.05$) than the controls. The plasma lactate in LTx patients increased earlier in exercise (Figure 4.1), which indicated a lower LT compared with controls. Plasma lactate elevation occurred at a workrate of 30.8 ± 2.8 watts in LTx compared with 79.2 ± 10.7 watts in control subjects ($P < 0.025$; Figure 4.2), or at 8.34 ± 0.7 $ml^{-1}\ kg^{-1}min^{-1}$ of $\dot{V}O_2$ peak in LTx and 16.7 ± 1.2 $ml\ min^{-1}\ kg^{-1}$ in controls ($P < 0.005$; Figure 4.3). LTx patients exhibited a low peak workrate, low $\dot{V}O_2$ peak and low HRpeak ($P < 0.05$; Table 4.3). Ventilatory limitation appeared to occur during exercise in one LTx patient with a measured ratio of maximal ventilation ($\dot{V}$Emax or $\dot{V}$Epeak) to predicted maximal voluntary ventilation (MVV) of 110% of predicted value. In the remainder, LTx patients $\dot{V}$Emax/MVV was < 82% of predicted value. The ratio of VEmax/MVV over 100% also occurred in 2 subjects in MC (105 and 116%; see Appendix IV). No patients or controls exhibited desaturation of blood oxygen at

the termination of exercise. In 6 of 7 patients leg fatigue was a predominant symptom at the termination of exercise whilst the remaining patient reported that leg fatigue and shortness of breath were equally severe when exercise ceased.

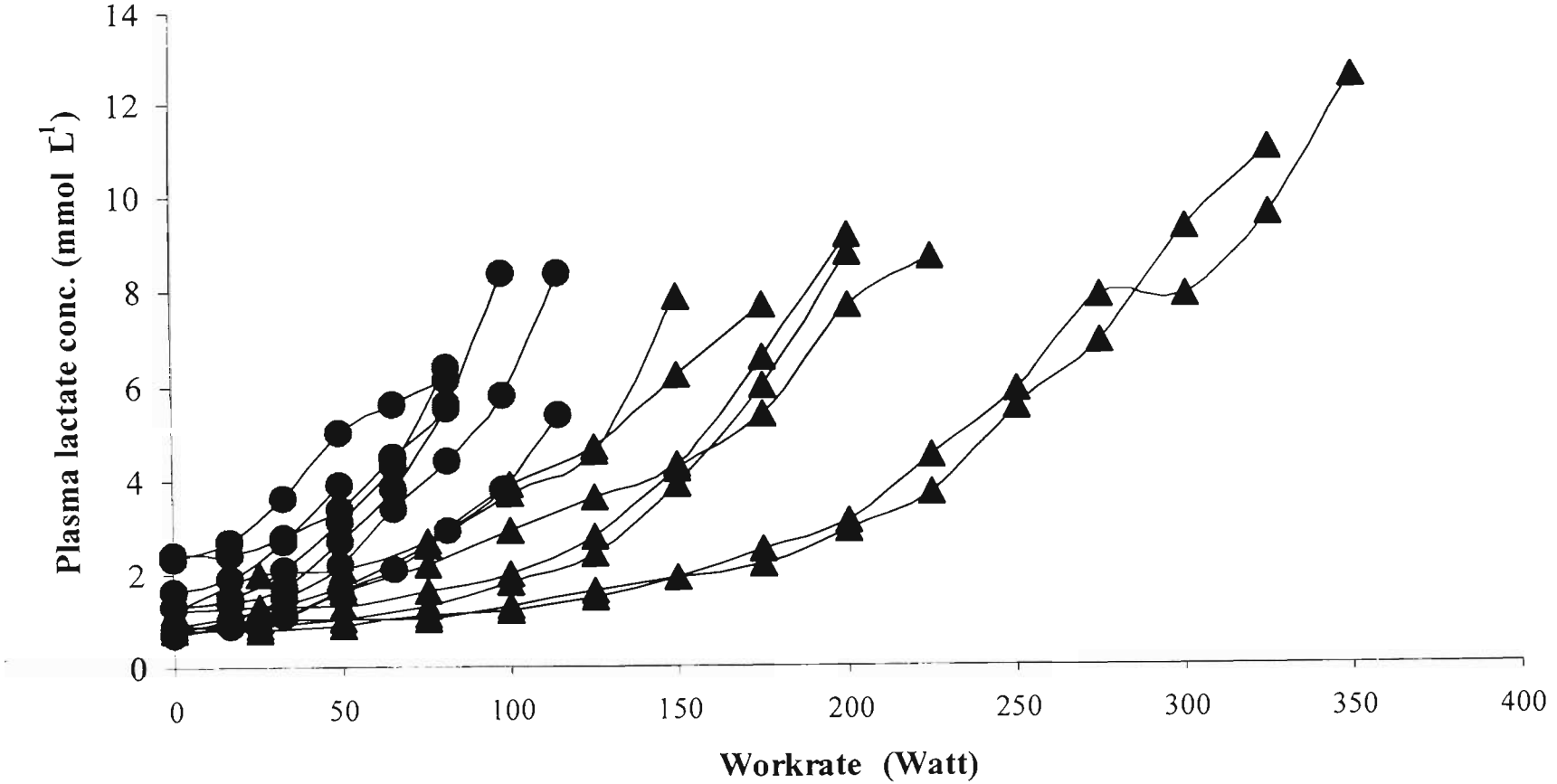


Figure 4.1. Plasma Lactate Levels in Increment Exercise

● LTx – lung transplant (n = 7); ▲ MC – age (sex)-matched controls (n = 7).

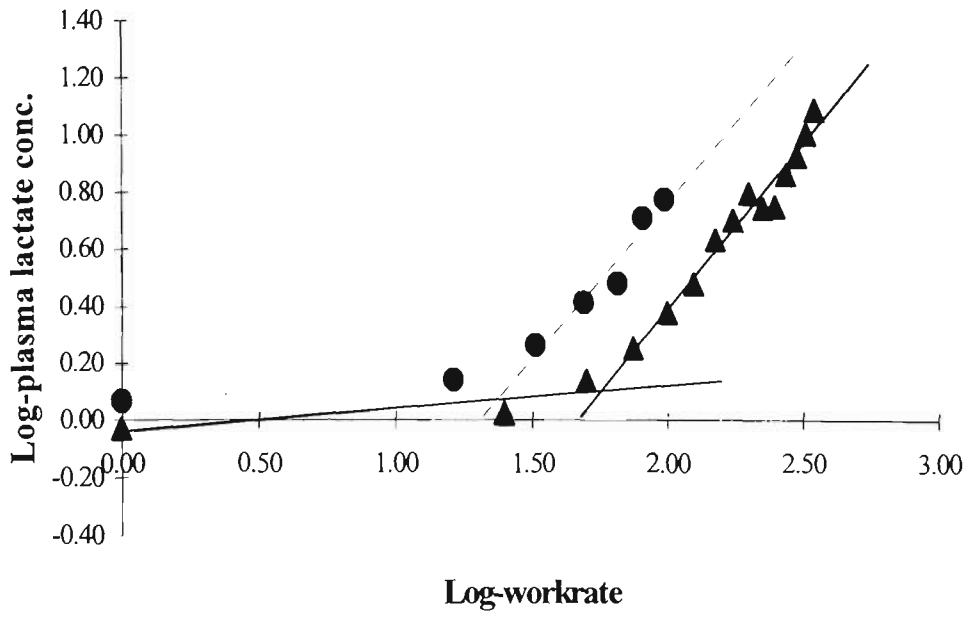


Figure 4.2. Lactate Threshold (Log plasma [Lactate]—Log workrate model)*

● LTx – lung transplantation (n=7); ▲ MC – age (sex)-matched controls (n=7); mean value; [Lactate], lactate concentration (mmol L^{-1}). * $P < 0.01$.

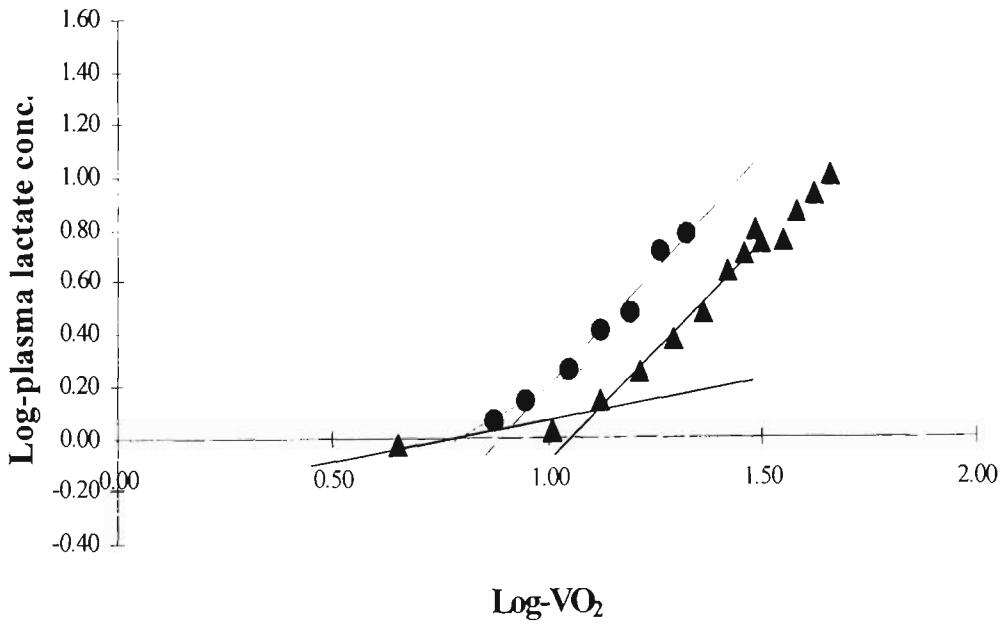


Figure 4.3. Lactate Threshold (log plasma [lactate]—log $\dot{V}O_2$ model)*.

● LTx – lung transplantation (n=7); ▲ MC – age (sex)-matched controls (n=7). Mean value; [Lactate], lactate concentration (mmol L^{-1}); * $P < 0.01$.

Table 4.3. Lung Function and Exercise Performance

	LTx	MC
Exercise duration (min)	$5.4 \pm 0.6^{\ddagger}$	9 ± 1
Peak workrate (W)	$88.5 \pm 10^{\S}$	217.9 ± 29.7
$\dot{V}$Epeak (L min^{-1})	$64.1 \pm 7.1^{\S}$	118.6 ± 12.9
MVV (L min^{-1})	$92.4 \pm 10.4^{\S}$	135.5 ± 5.34
VE/MVV(%)	72 ± 8	87 ± 7
$\dot{V}O_2$ peak (ml $kg^{-1}min^{-1}$)	$18.7 \pm 1.5^{\ddagger}$	36.9 ± 2.4
% predicted.	$56 \pm 3^{\dagger}$	111 ± 3
$\dot{V}CO_2$peak (ml $kg^{-1}min^{-1}$)	$22.9 \pm 2.8^{\S}$	44.7 ± 2.3
HR peak (bpm)	$137 \pm 6^{\S}$	177 ± 5
% predicted	$74 \pm 2^{\dagger}$	95 ± 2
LT (workrate, W)	$30.6 \pm 2.8^{\S}$	79.2 ± 10.7
LT($\dot{V}O_2$, ml $kg^{-1}min^{-1}$)	$8.34 \pm 0.7^{\S}$	$16.7 \pm 0.1.2$
SaO_2rest (mmHg)	97.9 ± 0.3	96.4 ± 0.4
SaO_2min (mmHg)	96.7 ± 0.7	94.5 ± 0.9

Values are mean ± SEM.. LTx, lung transplantation (n = 7); MC, age (sex)-matched controls (n = 7); $\dot{V}O_2$ peak, peak oxygen uptake; HR, heart rate; $\dot{V}$Epeak, minute ventilation at peak exercise; MVV, predicted maximum voluntary ventilation; LT, lactate threshold (log-log transformation of plasma lactate-workrate and plasma lactate-$\dot{V}O_2$ peak); SaO_2rest, resting oxygen saturation in blood gas; SaO_2min, minimum oxygen saturation in $\dot{V}O_2$ test; $^{*}P < 0.05$, $^{\dagger}P < 0.01$, compared with predicted values; $^{\ddagger}P < 0.05$, $\S$ $P < 0.01$, compared with age-matched controls.

4.3.2. Muscle Fibre Types

The average number of muscle fibre counted was 243 ± 29 versus 276 ± 34 (mean ± SEM) in LTx and controls, respectively. LTx recipients exhibited a lower proportion of type I muscle fibres (mean ± SEM; 24.9 ± 5.9% *vs.* 56.1 ± 2.4%, $P < 0.01$) (Figure 4.4).

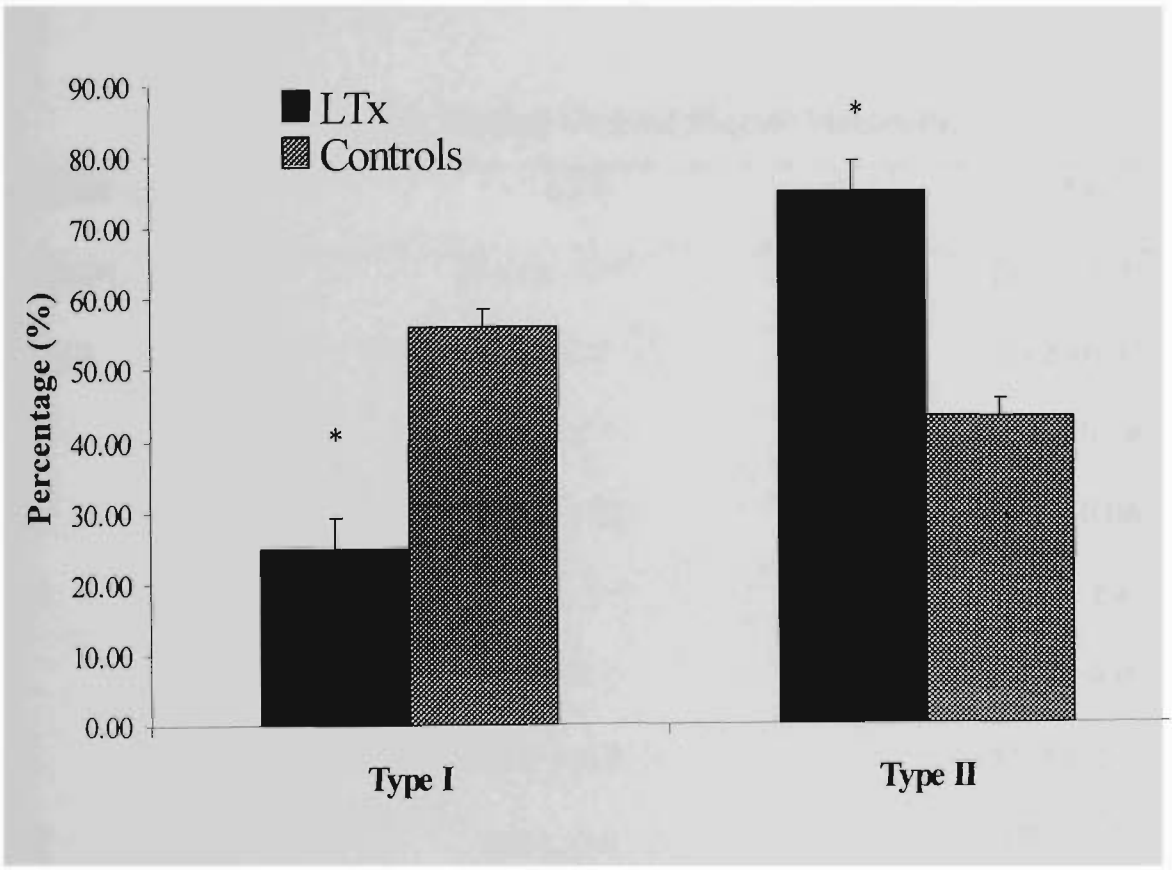


Figure 4.4. Proportion of Muscle Fibre Types

LTx, lung transplantation ($n = 7$); MC, age (sex)-matched controls ($n = 7$); mean ± SEM.

* $P < 0.01$.

4.3.3. Resting Muscle Metabolites

In resting skeletal muscle, lactate and IMP concentrations were significantly higher, and ATP was significantly lower in LTx compared with controls ($P < 0.01$). The concentrations of muscle Cr, PCr, ADP, AMP and total creatine (TCR, Cr + PCr) did not differ significantly between the two groups. The total adenine nucleotides (TAN, ATP + ADP + AMP) and the ratio of ATP/ADP were significantly lower in LTx than controls ($P < 0.01$; Table 4.4).

Table. 4.4. Resting Skeletal Muscle Metabolites

Metabolites	LTx	MC
ATP (HPLC)	21.4 ± 1.2**	26.0 ± 1.3
ATP (E)	22.5 ± 0.8 *	25.2 ±0.9
ADP	2.81 ± 0.19	2.41 ± 0.14
AMP	0.12 ± 0.02	0.24 ± 0.06
TAN	24.3 ± 1.3**	28.6 ± 1.4
Cr	55.0 ± 4.5	46.9 ± 4.0
PCr	96.8 ± 5.0	93.2 ± 2.7
TCR	152 ± 7.5	140 ± 5.3
Glycogen	461± 72	462 ± 35
IMP	0.26 ± 0.04**	0.05 ± 0.01
Lactate	16.3 ± 10**	8.4 ± 0.9
ATP/ADP	7.67 ± 0.3**	10.9 ± 0.7

Values are mean ± SEM, expressed as mmol kg^{-1} dry weight of muscle, except ATP/ADP (%). LTx, lung transplantation ($n = 7$); MC, age (sex)-matched controls ($n = 7$); ATP (HPLC), measured by HPLC; ATP (E), ATP measured enzymaticly; TCR, total creatine (PCr + Cr); TAN (determined by HPLC), total adenine nucleotides (ATP + ADP + AMP). The ATP/ADP ration was calculated from HPLC determined ATP and ADP. * $P < 0.05$; ** $P < 0.01$.

4.3.4. Enzyme Activity

LTx recipients demonstrated lower activity of the mitochondrial enzymes CS, GDH, KGDH and HAD per muscle weight ($P < 0.005$) and higher activity in the glycolytic enzyme, PFK, per muscle weight compared with controls ($P < 0.05$). There were no differences in the activity of PHOSPH, HK and PK per muscle weight between the two groups (Table 4.5). The average value of muscle protein percentage in the LTx patients was as the controls (15% ± 1% *vs.* 15% ± 1%). When the activity of enzymes was expressed per unit muscle protein, the mitochondrial enzyme activities were still higher than LTx patients ($P < 0.005$). The activity of PHOSPH, HK and PK was still not different between the two groups. The activity of PFK, however, tended to be higher than LTx group ($P = 0.0628$). These results are listed in Table 4.6.

Table 4.5. Muscle Enzyme Activity Expressed Per Unit Muscle Weight

Enzyme	LTx	MC	% difference
GDH	0.56 ± 0.04**	0.97 ± 0.06	-42
CS	10.2 ± 1.5**	18.7 ± 1.5	-45
KGDH	0.39 ± 0.07**	1.01 ± 0.08	-61
HAD	2.66 ± 0.2**	4.7 ± 0.3	-43
PFK	36.2 ± 2.9*	28.1 ± 1.6	+29
PHOSPH	15.9 ± 1.9	14.3 ± 1.0	+11
HK	1.66 ± 0.2	1.64 ± 0.2	+1
PK	234 ± 29.8	262 ± 13.2	-11

Values are mean ± SEM, expressed as μmol min^{-1} g^{-1} wet weight of muscle. LTx, lung transplantation ($n = 7$); MC, age (sex)-matched controls ($n = 7$); % difference, [(LTx-MC)/MC %]. * $P < 0.05$; ** $P < 0.005$.

Table 4.6. Muscle Enzyme Activity Expressed Per Unit Muscle Protein

Enzyme	LTx	MC	% difference
GDH	3.96 ± 0.38**	6.77 ± 0.55	-42
CS	71.7 ± 10.8**	130.6 ± 13.2	-46
KGDH	2.68 ± 0.5**	6.99 ± 0.6	-62
HAD	18.5 ± 1.5**	33.1 ± 3.1	-44
PFK	251 ± 20.0	196 ± 17.8	+28
PHOSPH	108 ± 9.8	101 ± 10.5	+9
HK	11.3 ± 1.2	11.4 ± 1.0	-0.8
PK	1600 ± 186	1864 ± 224	-14

Values are mean ± SEM, expressed as mmol min^{-1} g^{-1} protein of muscle. LTx, lung transplantation (n = 7); MC, age (sex)-matched controls (n = 7); % difference, (LTx-MC)/MC %. * $P < 0.05$; ** $P < 0.005$.

4.3.5. MAPR

Mitochondrial suspension protein yield (mitochondrial suspension protein content/muscle weight, g protein kg^{-1}) was significantly lower (~43%) in LTx compared with controls ($P <$ 0.05). Protein concentration (mg ml^{-1}) in mitochondrial suspension, mitochondrial protein in the assay cuvette (ng ml^{-1}) and the yield of mitochondria were not statistical different between the two groups ($P > 0.05$, Table 4.7). MAPR in the presence of P + M, PC + M, and α-KG were significantly lower in LTx than controls when MAPR expressed per unit mitochondrial suspension protein (P <0.05, Table 4.8). There was a tendency for a lower MAPR in the presence of PPKM expressed per unit protein of mitochondrial suspension in the LTx (P = 0.053). MAPR in the presence of S + R was similar in the two groups. When expressed per unit muscle weight, MAPR, in the presence of all substrates, were significantly lower in LTx than controls ($P < 0.05$, Table 4.9).

Table 4.7. Mitochondrial Suspension Protein and Mitochondrial Yield

	LTx	MC
Muscle weight.(mg)	37.0 ± 3.1	32.3 ± 1.2
Protein concentration of mit. suspension (mg ml^{-1}).	0.42 ± 0.1	0.62 ± 0.1
Mit. protein in assays (ng ml^{-1}).	16.9 ± 3.7	24.9 ± 3.4
Yield of mit. suspension protein in muscle (g kg^{-1}).	2.26 ± 0.4*	3.94 ± 0.4
Yield of mit. from GDHim /GDHt (%)	21.5 ± 3.3	22.7 ± 2.2

Values are mean ± SEM. LTx, lung transplantation (n = 7); MC, age (sex)-matched controls (n = 7); mit., mitochondria; GDHim, intact mitochondrial GDH activity; GDHt, total GDH activity. * $P < 0.025$.

Table 4.8. MAPR Expressed Per Unit Protein of Mitochondrial Suspension

Substrates	LTx	MC	% difference
P + M	0.24 ± 0.04**	0.37 ± 0.02	-35
PC + M	0.16 ± 0.02**	0.30 ± 0.02	-47
S + R	0.32 ± 0.05	0.37 ± 0.04	-14
A-KG	0.21 ± 0.05*	0.4 ± 0.03	148
PPKM	0.40 ± 0.09	0.63 ± 0.03	-37

Values are mean ± SEM, expressed as mmol min^{-1} g^{-1} protein of mitochondrial suspension. LTx, lung transplantation (n = 7); MC, age (sex)-matched controls (n = 7). % difference, (LTx-MC)/MC %. * $P < 0.05$. ** $P < 0.005$.

Table 4.9. MAPR Expressed Per Unit Muscle Weight

Substrates	LTx	MC	% difference
P + M	2.63 ± 0.56**	6.29 ± 0.53	-58
PC + M	1.71 ± 0.27**	4.97 ± 0.31	-66
S + R	3.45 ± 0.7*	6.25 ± 0.71	-45
A-KG	2.3 ± 0.59**	6.72 ± 0.65	-66
PPKM	4.42 ± 1.12**	10.6 ± 0.73	-58

Values are mean ± SEM, expressed as mmol min^{-1} g^{-1} weight of muscle. LTx, lung transplantation (n = 7); MC, age (sex)-matched controls (n = 7). % difference, (LTx-MC)/MC %. * $P < 0.05$. ** $P < 0.005$.

4.3.6. Regressions

When the data of LTx patients and controls were pooled together, MAPR in the presence of all substrates showed significant positive linear regressions with $\dot{V}O_2$ peak, the proportion of type I fibre, oxidative enzymes and ratio of ATP/ADP ($P < 0.05$). Examples of regressions are shown in Figure 4.5a-d. There were significant positive regressions between the proportion of type I fibres and $\dot{V}O_2$ peak, oxidative enzymes and the ATP/ADP ratio ($P < 0.05$). The resting muscle IMP and lactate concentrations, in contrast, showed significant negative regressions with MAPR in the presence of various combinations of substrates, the ratio of ATP/ADP and the proportion of type I fibres ($P < 0.05$, Table 4.10). Examples are shown in Figure 4.6a-d. Examples of R values for these regressions are listed in Table 4.10.

Table 4.10. Regressions Values between the MAPR and $\dot{V}O_2$ peak, Type I Fibre, Oxidative Enzymes, and Metabolites

	$\dot{V}O_2$	Type I	PPKM	S + R	A-KG	PC-M	P + M	CS	GDH	KGDH	HAD	ATP/ADP	IMP
Type I	0.71†												
PPKM	0.79†	0.78†											
S + R	0.67†	0.74†	0.89†										
A-KG	0.72†	0.76†	0.90†	0.88†									
PC + M	0.78†	0.79†	0.90†	0.75†	0.94†								
P + M	0.74†	0.73†	0.91†	0.77†	0.85†	0.91†							
CS	0.79†	0.47	0.78†	0.64*	0.70†	0.75†	0.83†						
GDH	0.86†	0.67†	0.85†	0.67†	0.74†	0.83†	0.89†	0.92†					
KGDH	0.81†	0.62*	0.77†	0.59*	0.72†	0.82†	0.87†	0.92†	0.93†				
HAD	0.74†	0.86‡	0.74†	0.61*	0.72†	0.81†	0.77†	0.66†	0.83†	0.79†			
ATP/ADP	0.59*	0.59*	0.57*	0.54*	0.63*	0.63*	0.58*	0.68†	0.62*	0.66*	0.68†		
IMP	-0.76†	-0.79†	-0.76†	-0.71†	-0.75†	-0.80†	-0.73†	-0.61*	-0.70†	-0.57*	-0.78†	-0.71†	
La-	-0.69†	-0.83†	-0.79†	-0.65*	-0.77†	-0.85†	-0.79†	-0.72†	-0.81†	-0.84†	-0.88‡	-0.80†	0.68†

Data is the pools of the lung transplantation (LTx) patients (n = 7) and age (sex)-matched controls (MC) (n = 7). * P <0.05; † P < 0.01.

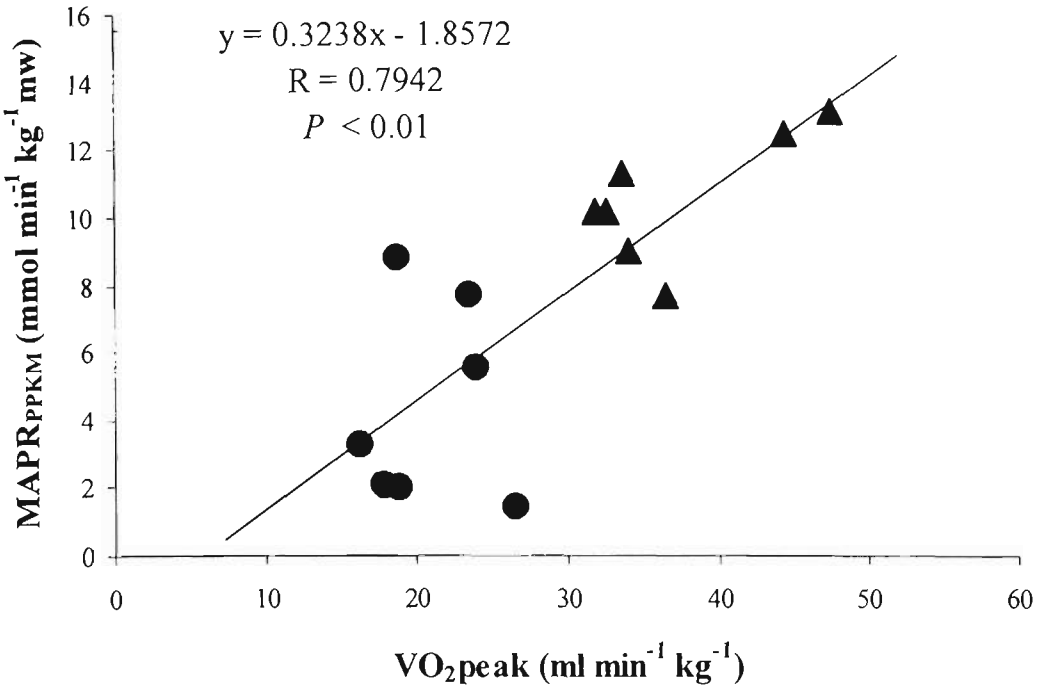


Figure 4.5-a. Regression between MAPR with PPKM and $\dot{V}O_2$ peak

● lung transplantation patients (n = 7); ▲ age (sex)-matched controls (n = 7); mean value.

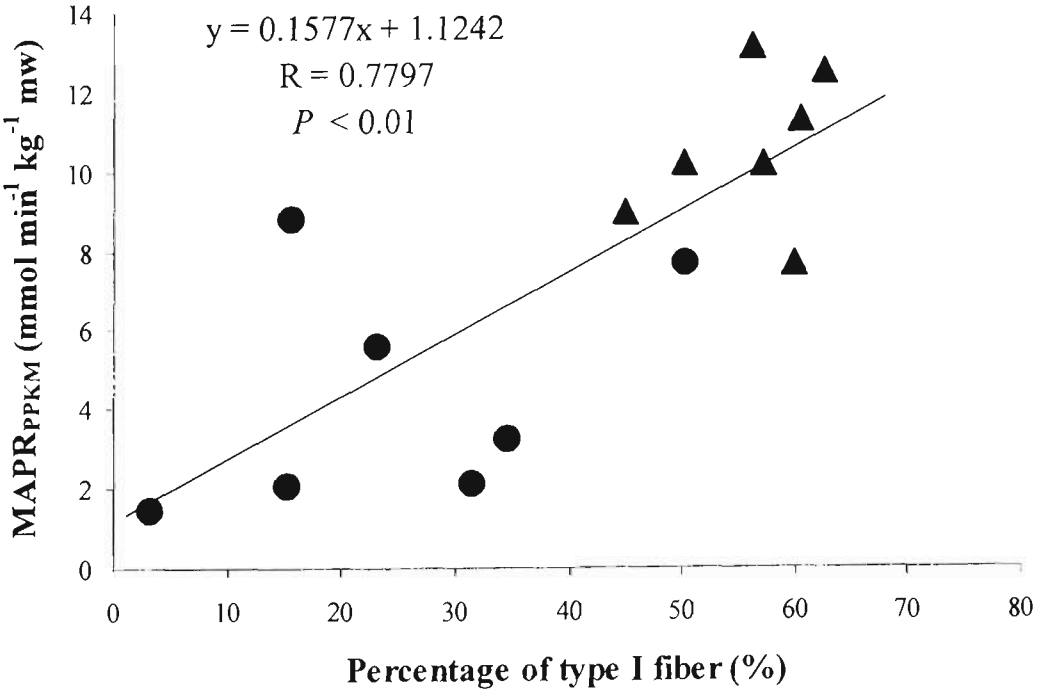


Figure 4.5-b. Regression between MAPR with PPKM and Type I Fibres

● lung transplantation patients (n = 7); ▲ age (sex)-matched controls (n = 7); mean value.

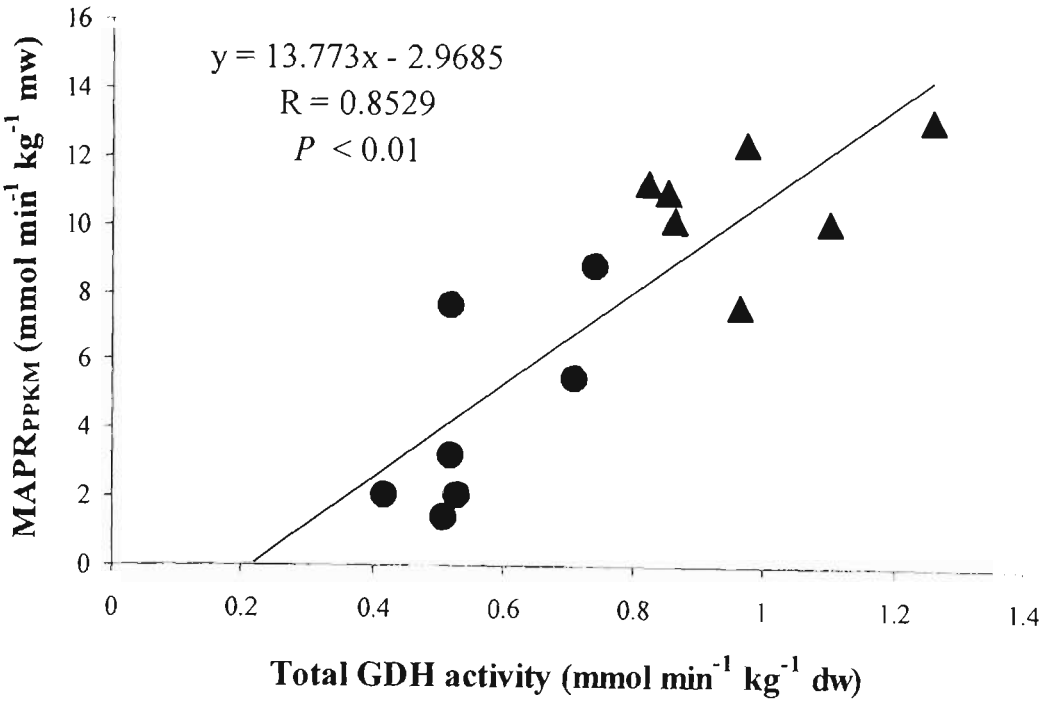


Figure 4.5-c. Regression between MAPR with PPKM and GDH Activity

● lung transplantation patients (n = 7); ▲ age (sex)-matched controls (n = 7); mean value.

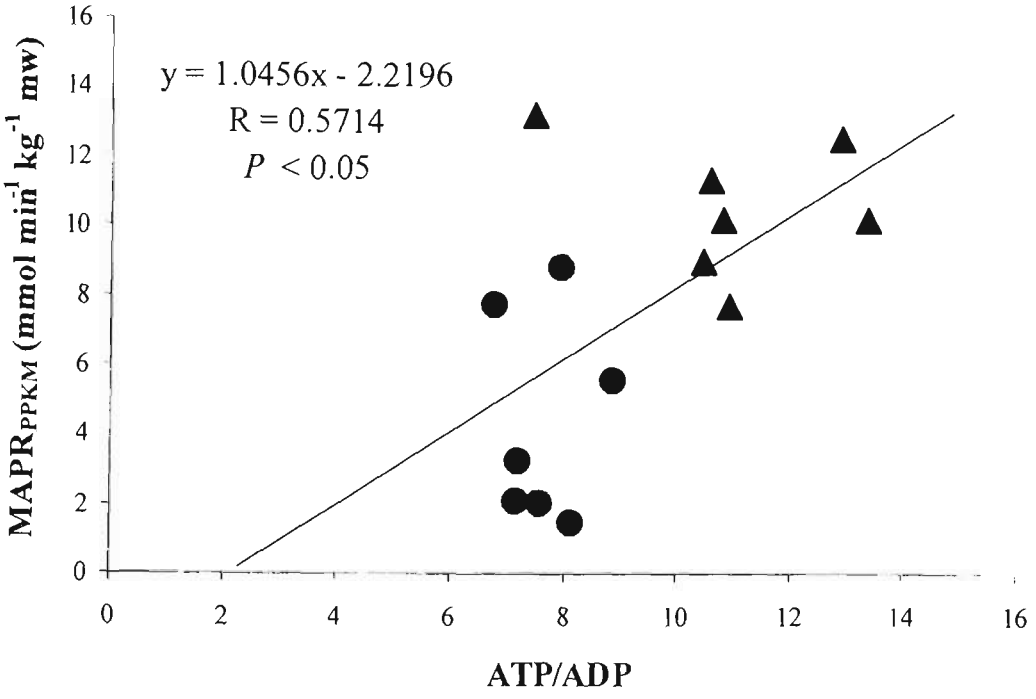


Figure 4.5-d. Regression between MAPR with PPKM and Ratio of ATP/ADP

● lung transplantation patients (n = 7). ▲ age (sex)-matched controls (n = 7); mean value.

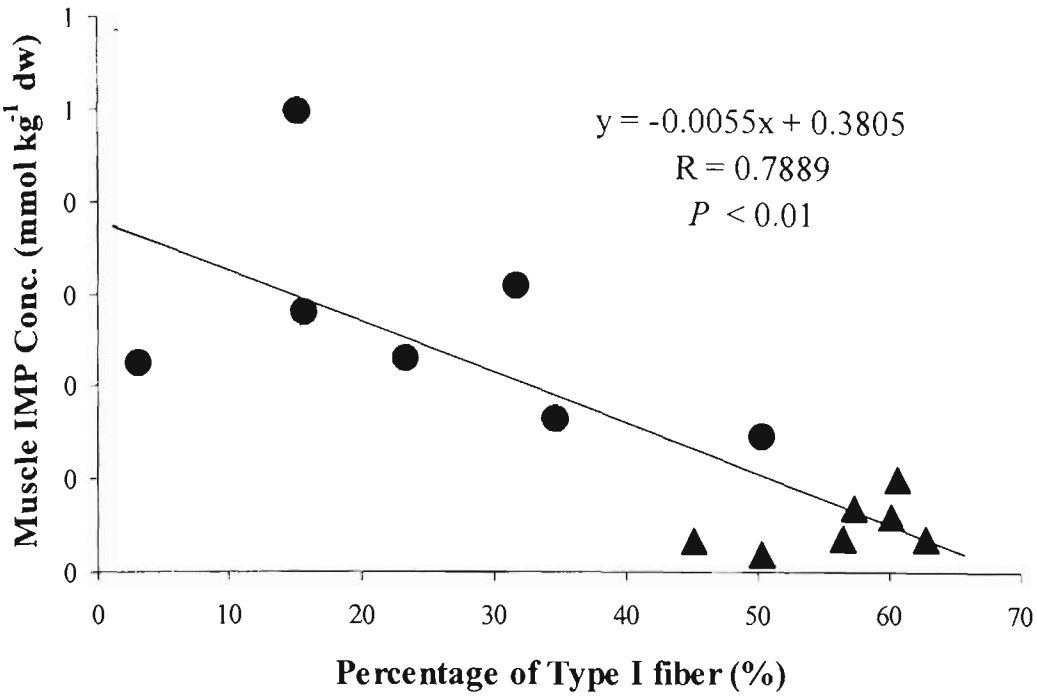


Figure 4.6-a. Regression between IMP and Type I Fibres

● lung transplantation patients (n = 7); ▲ age (sex)-matched controls (n = 7); mean value.

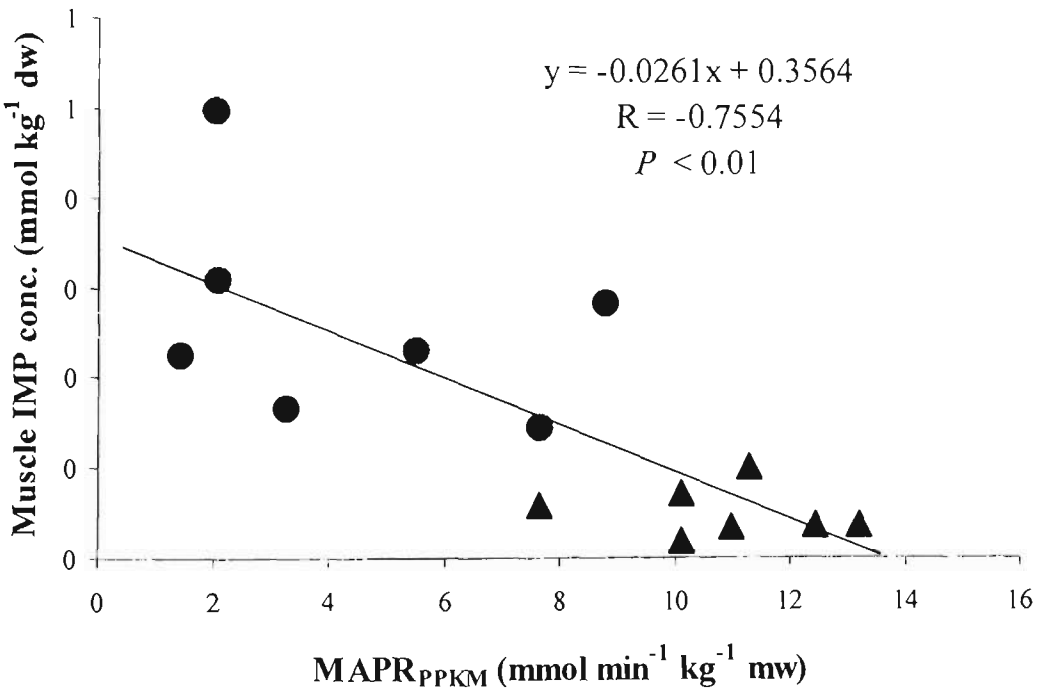


Figure 4.6-b. Regression between IMP and MAPR with PPKM

● lung transplantation patients (n = 7); ▲ age (sex)-matched controls (n = 7); mean value.

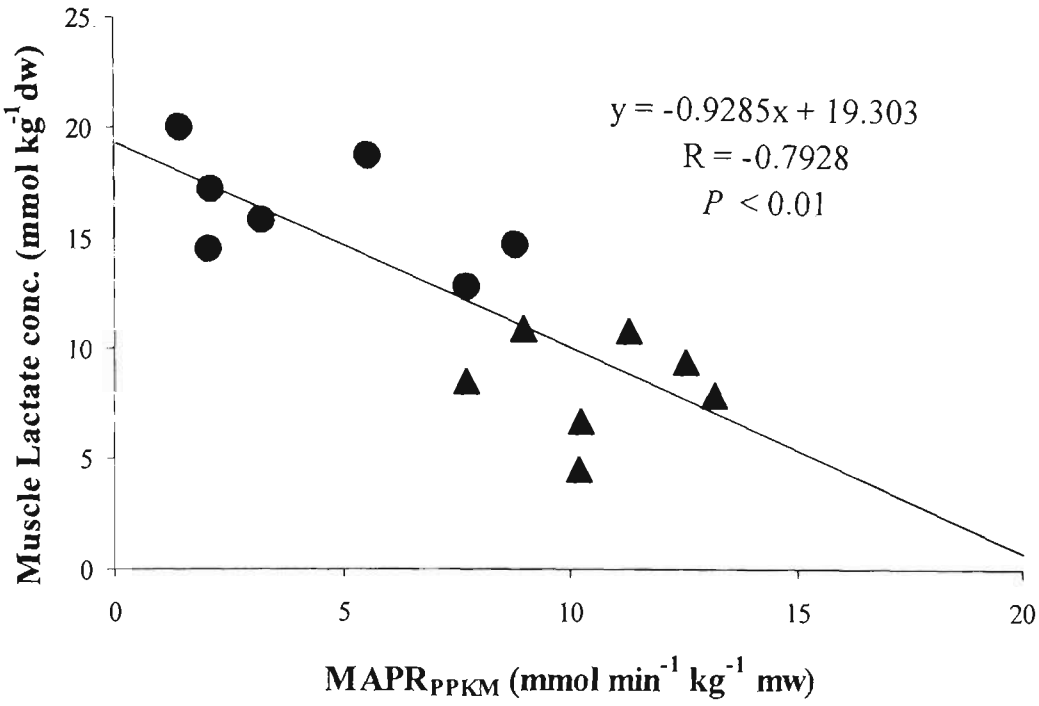


Figure 4.6-c. Regression between Muscle Lactate and MAPR with PPKM

● lung transplantation patients (n = 7); ▲ age (sex)-matched controls (n = 7); mean value.

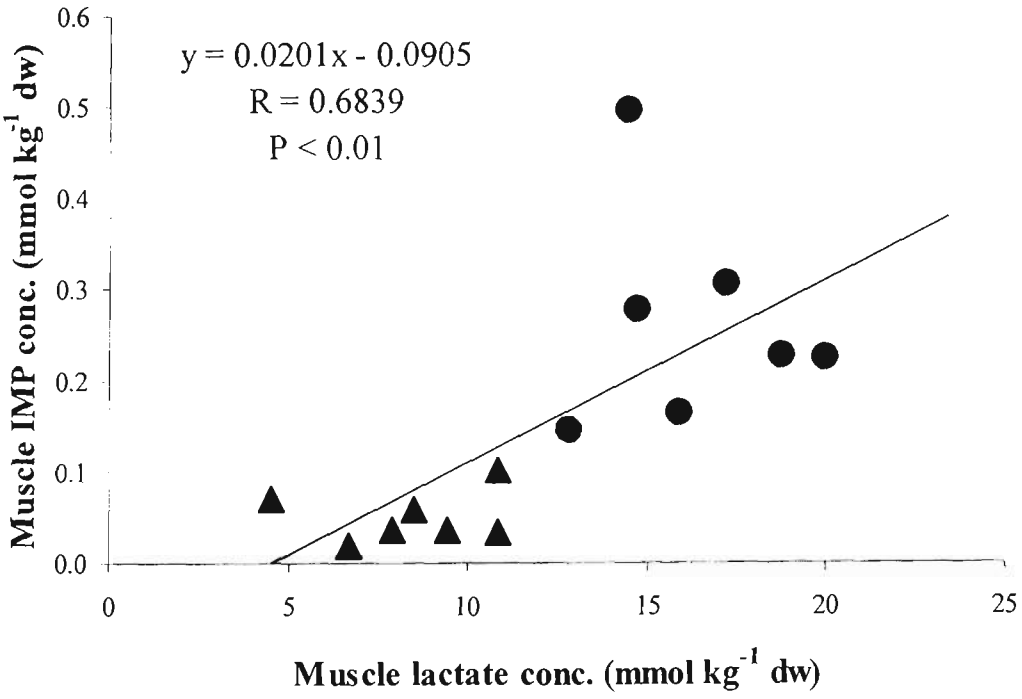


Figure 4.6-d. Regression between Muscle Lactate and IMP

● lung transplantation patients (n = 7); ▲ age (sex)-matched controls (n = 7); mean value.

4.4. DISCUSSION

4.4.1. Main Findings in this Study

The major findings of this study were that LTx recipients had a lower exercise capacity indicated by a shorter duration exercise and a lower $\dot{V}O_2$ peak during the incremental exercise test compared to normal matched controls. They also exhibited an earlier rise in plasma lactate during these tests. Although the LTx recipients demonstrated a mild mixed obstructive/restrictive ventilatory defect, only 1 of 7 LTx recipients experienced ventilation limitation during exercise and none showed any desaturation in blood oxygen. Secondly, LTx patients had a lower proportion of type I fibres, lower ATP content and higher lactate and IMP in the vastus lateralis muscle at rest compared with the controls. Thirdly, LTx patients had a lower activity of oxidative enzymes and higher activity of PFK in skeletal muscle than the controls. Finally, the LTx recipients had reduced skeletal muscle MAPR in the presence of a range of substrates, both when expressed per unit muscle mass and per unit protein of mitochondrial suspension.

4.4.2. Muscle Fibre Type

The marked alteration in muscle fibre type proportion is likely to be a major contributor to the reduction of muscle oxidative capacity and exercise tolerance in LTx. In general, type I fibres are fatigue resistant, and rich in mitochondria with high levels of oxidative enzymes. Type II fibres (particularly type IIb fibres) have fewer mitochondria and tend to be better suited to anaerobic metabolism possessing increased glycolytic capabilities (see Section 2.3.1). In addition, the mitochondria present in different muscle fibre types may have different oxidative function. Jackman and Willis (1996) demonstrated that mitochondrial State 3 respiration rates

in the presence of P + M and PC + M were higher in the mitochondria from rabbit soleus muscle, which is composed of 98% type I fibres, compared with the mitochondria from gracilis muscle (99% type IIb fibres). Therefore, in LTx recipients, a shift of type I to type II fibres would reduce the oxidative capacity in skeletal muscle, and may be associated with an increased reliance upon anaerobic metabolism.

The alteration of muscle types in LTx recipients may have occurred pre-transplantation. This possibility is supported by the observation that patients with COPD who may be candidates for lung transplantation, have a low proportion of type I fibres in skeletal muscle (Jakobsson et al 1990; Maltails et al 1996; Whittom et al 1998). The cause of this fibre type alteration is not known, but is possibly due to the muscle deconditioning after prolonged bed-rest, inactivity (see Section 2.3.4.1) and/or hypoxaemia. Morton et al (1997) demonstrated that patients with COPD had a low proportion of type I fibres in vastus lateralis muscle (36%; normal ~50%) and high proportion type I fibres in the diaphragm (68%) and the intercostal (70%) muscles. Generally, diaphragm and intercostal muscles in humans consist of ~ 50% and 60% type I fibres, respectively (Mizuno 1991). This study suggested that muscle activity play a key role for changes in the proportions of muscle fibre types in skeletal muscles. Whittom et al (1998), however, demonstrated that a period exercise training (12 weeks) increased the cross-sectional area in all muscle fibre, but did not change the proportion of muscle type fibres. This indicates that the inactivity may not be the only cause for muscle fibre proportion alteration. Jakobsson et al (1990) and Hildebrand et al (1991) demonstrated that the COPD patients the lower arterial PO_2 the lower proportion of muscle type I fibres, indicating that hypoxaemia may be one cause for fibre type alteration. The long-term bed rest, physical inactivity and hypoxaemia would have been the conditions common to all LTx patients in this study prior to transplant. Thus, the low proportion of type I fibres in LTx patients may has occurred pre-transplant and persist post transplantation in the present study. There are no longitudinal studies of the alteration in muscle fibre types in LTx patients. Such a longitudinal

study has been conducted in cardiac transplant patients (Bussiéres et al 1997). The patients in that study, had a low proportion of the type I fibres and this situation was not reversed after 12 months successful heart transplantation.

4.4.3. Muscle Metabolism

Accumulation of lactate in the resting skeletal muscle of LTx reflects a high tendency for glycolysis and/or reduced muscle mitochondrial oxidative phosphorylation. The high proportion of type II fibres may also contribute to an increase in lactate concentration in the resting muscle of LTx because type II fibres have high LDH activity (Essén and Henrikson 1980) and high glycolytic capacity (Lowry et al 1978). The present group of transplant recipients had a low ATP concentration with a normal level of muscle PCr in resting muscle. Low muscle ATP levels have also been observed in patients with COPD (Jakobsson and Jorfeldt 1995). The patients in the previous study have hypoxaemia which potentially could be a cause of the low ATP in muscle. The patients in this study, however, did not have hypoxaemia when they participated in exercise tests, despite having lower FEV_1 and VC than predicted values and, hence the low ATP must have some other cause. Muscle fibre alteration in the present study was not consistent with the change of ATP in resting muscle because the ATP concentration in type II fibres is usually 10% higher than in type I fibres (Sant'Ana Pereira et al 1996). Therefore, low ATP in the resting muscle of the LTx patients in the present study may result from a low capacity for mitochondrial ATP synthesis. The contention that mitochondrial dysfunction is responsible for the reduced ATP level in resting muscle is also supported by the PCr levels. The PCr concentration in resting human skeletal muscle is 15%-20% higher in type II fibres compared with type I fibres (Söderlund and Hultman 1991, Sahlin et al 1997). Hence, normal muscle PCr levels in LTx group, despite elevated proportion of type II fibres, may also indicate impaired mitochondrial function.

The LTx patients in the present study also exhibited a low ATP/ADP ratio. The ratio of ATP/ADP is viewed as an indicator of the efficiency of the transfer from energy producing processes to utilising processes in cells (Wilson 1994). A low ATP concentration and a decreased ratio of ATP/ADP can activate the enzyme AMP deaminase (Newsholme and Start 1973), which catalyses the conversion of AMP to IMP and ammonia (NH_3) (Lowenstein 1972; Jansson et al 1987). Normally, IMP content of resting muscle is very low, but increases significantly in skeletal muscle during high intensity exercise (Stathis et al 1994), at rest in ischaemia (Norman et al 1991) and in patients with severe impairment of lung function (Pouw et al 1998). It is generally believed that elevated IMP indicates a disturbed balance of energy metabolism in muscle (Norman 1994). Hence, mildly but significantly elevated IMP in the resting muscle of LTx may be due to decreased ATP regeneration from oxidative phosphorylation in mitochondria.

This study has shown that there are significant negative regressions between muscle IMP, lactate and MAPR. Muscle IMP and lactate also negatively correlated with the proportion of type I fibres (Table 4.10). These findings suggest that the metabolic abnormalities observed in skeletal muscle of LTx patients are most likely to be secondary to impaired mitochondrial function and the increased proportion of type II fibres.

4.4.4. Metabolic Enzyme Activities

Low activity of oxidative enzymes has been demonstrated in LTx patients in the present study. The oxidative enzymes CS and KGDH (or 2-oxoglutarate dehydrogenase) catalyse reactions in the mitochondrial TCA cycle (see Section 2.2.2.3 and Figure 2.8). Lower activity of CS and KGDH may limit metabolism in the TCA cycle in the LTx patients. A low activity of the β-oxidation enzyme HAD in LTx patients, indicates a reduced mitochondrial capacity for fatty acid oxidation. The enzyme GDH catalyses the conversion of glutamate to α-

ketoglutarate in the mitochondrial matrix (see Section 2.2.2.2 and Figure 2.7). A lower activity of GDH at least partially indicates that the metabolic capacity for amino acid metabolism in mitochondria was lower in LTx than in controls.

The reduced levels of CS and HAD activities in LTx observed in the present study are similar to the observations of Jakobsson et al (1995) and Maltais et al (1996) in patients with COPD. In addition, a decreased activity of muscle KGDH in LTx was similar to the study for skeletal muscle KGDH in CHF patients (Näveri et al 1997). The GDH activity, however, has been reported in neither LTx patients nor in heart and lung disease patients. The increased glycolytic enzyme (PFK) activity indicates that skeletal muscle in LTx patients is adapted to anaerobic metabolism when mitochondrial oxidation is impaired. The LTx patients in the present study had a 29% higher activity of PFK than the controls. This is similar to a 34% higher activity of PFK in skeletal muscle of patients with COPD compared with controls reported by Jakobsson et al (1995). The higher muscle PFK activity in LTx patients may help to explain the increased lactate concentration in resting muscle in the present study. The reduced oxidative enzyme activity and elevated PFK activity in LTx patients are partially related to the alteration in muscle fibres (see Section 2.3.1).

4.4.5. MAPR

As mentioned in Section 2.2.3.3, MAPR in the presence of pyruvate, malate and α-ketoglutarate is an indication of the capacity for carbohydrate metabolism in mitochondria and the functional integrity of Complex I. MAPR using succinate and rotenone (S + R) indicates the capacity of Complex II. Palmitoyl-carnitine, as substrate in MAPR determination, reflects the capacity for fatty acid oxidation in mitochondria. This substrate may also indicate the function of Complex I and Complex III (see Section 2.2.2.4). Compared to normal matched controls, LTx recipients exhibited an impaired *in vitro* MAPR in the

presence of all substrates. Therefore, the LTx patients have lower mitochondrial metabolic capacity for carbohydrate and fatty acid oxidation in skeletal muscle. These low values for MAPR indicated the LTx patients have impaired functions in Complexes I, II and III of the mitochondrial electron transfer chain in skeletal muscle. The low muscle mitochondrial function in the LTx may be one of key factors resulting in alteration of skeletal muscle metabolism and exercise limitation in the LTx patients.

4.4.6. Potential Causes of Reduction in Mitochondrial Capacity in LTx Patients

A low mitochondrial volume or reduced mitochondrial numbers may be the cause of reduced mitochondrial function in the LTx patients as indicated by MAPR expressed per unit muscle weight. The present study, however, did not investigate the mitochondrial volume and numbers in skeletal muscle. Nevertheless, the lower level of protein in the mitochondrial suspension in LTx compared with controls indicates, albeit indirectly, a reduction in mitochondrial volume and/or numbers in skeletal muscle in the LTx patients. Mitochondrial function in the LTx may also due to the impairment of individual mitochondrial function. The functional impairment of the individual mitochondria is indicated by low MAPR *in vitro* expressed per unit mitochondrial suspension protein in this study. It is possible, therefore, that the LTx patients have a reduced mitochondrial volume or numbers and impaired function of individual mitochondria in skeletal muscle. As previously indicated, transformation from type I to type II muscle fibre is an important factor which will contribute to the reduced the mitochondrial capacity in LTx patients. The low activity of oxidative enzymes will also affect mitochondrial function in the LTx patients.

The causes of reduced MAPR and oxidative enzyme activity in the LTx patients, observed in the present study, are not clear. Previous studies have demonstrated that altered mitochondrial

capacity in skeletal muscle is caused by a numbers of factors including muscle disuse (Max 1972; Yajid et al 1998), immobilisation (Kauhanen et al 1993) and physical deconditioning (Ferretti et al 1997). Reduction of mitochondrial capacity in skeletal muscle, as indicated by low oxidative enzyme activity, is also observed in severe chronic pulmonary disease (Jakobsson et al 1995). Subsequently, it has been demonstrated that supplementary oxygen for patients with COPD significantly increased the skeletal muscle PCr/(PCr + Cr) ratio indicating improved oxidative metabolism (Jakobsson and Jorfeldt 1995). Examination of skeletal muscle metabolism in patients with COPD using ^{31}P NMR has also shown that oxygen supplementation significantly reduced the calf muscle intracellular acidosis and Pi/PCr ratio at the end of exercise of performance 360 flexions of foot again indicating improved oxidative metabolism (Payen et al 1993). This would support the contention, therefore, that hypoxaemia might be an important factor causing the reduction of skeletal muscle mitochondrial function in COPD. In the present study, hypoxaemia can not account for the similar reduction in the LTx patients because the blood oxygen saturation was normal in these patients. Hence, other factors must be the cause of impaired mitochondrial function in skeletal muscle of the LTx patients.

Long-term treatment with immunosuppressive drugs, such as PNL, can cause skeletal muscle histological abnormalities and reduce mitochondrial function post transplantation (see Section 2.4.3.2). Previous studies have demonstrated corticosteroids cause muscle wasting (Nava et al 1996) and muscle type II fibre atrophy in rats (Wilcox et al 1989; Nava et al 1996). These steroids also induce acute and chronic corticosteroid myopathy in humans (Bowyer et al 1985; Williams et al 1988; Shee 1990; Decramer et al 1996). In terms of mitochondrial function, these agents can inhibit mitochondrial oxygen consumption (Lettéron et al 1997; Simon et al 1997) and reduce the activity of mitochondrial fatty acid enzymes in mice (Lettéron et al 1997). The main effects of corticosteroids on skeletal muscle changes and on mitochondrial function have been described in Section 2.4.3.2.

It has been demonstrated that CyA inhibits kidney mitochondria respiration in human (Jung and Reinholdt 1987) in a dose-dependent manner. Inhibition of O_2 consumption in mitochondria isolated from rat skeletal muscle has been reported in response to a high concentration of CyA (25 $\mu g\ ml^{-1}$) added to the test chamber (Hokanson et al 1995). In addition, mitochondria isolated from the skeletal muscle of rats fed high dosages of CyA (20 $mg\ kg^{-1}$ per day for 14 days) were similarly affected (Mercier et al 1995). The latter report showed a significant linear regression between endurance exercise time in rats and impaired mitochondrial State 3 respiration. The study by Hokanson et al 1995, however, demonstrating *in vitro* CyA inhibition of skeletal muscle mitochondrial function, used far higher concentrations of CyA (25 $\mu g\ ml^{-1}$) than clinically maintained plasma CyA levels (Baumgartner et al 1987). The effect of low concentration of CyA (1 $\mu g\ ml^{-1}$) on mitochondrial ATP production rate will be examined in Chapter 5 of this thesis.

Recently, Simon et al (1997) have demonstrated that a combination of PNL and AZA along with CyA had a more severe impact on renal mitochondrial function than that induced by CyA alone. Since 6/7 LTx patients were receiving the combination of CyA, PNL and AZA therapy, it is possible that these drugs contribute significantly to the impaired mitochondrial function observed in this study.

The mechanism by which CyA inhibits mitochondrial function are still not known. Hokanson et al (1995) have suggested that CyA may have no direct affect on the components of the ECT. These authors proposed that CyA may influence mitochondrial function through its effects on the membrane permeability transition pore (MPTP) in the inner mitochondria membrane. CyA may block this pore which controls Ca^{2+} passage across the inner mitochondrial membrane, but the consequences of this effect on mitochondrial function are not clear. Some reports have indicated that CyA inhibits Ca^{2+} efflux via the MPTP leading to increased mitochondrial Ca^{2+} concentration and decreased mitochondrial function (Salducci et al 1996). In contrast, other data has demonstrated beneficial or protective effects of CyA

inhibition of the MPTP by reducing the damage caused by anoxia and oxidant stress injury or ischaemia reperfusion damage (Griffiths and Halestrap. 1993). These possible beneficial effect, however, is not consistent with observation of that CyA has been shown to enhance the generation of the ROS *in vitro* from cell cultures (Zamzami et al 1995; Wolf et al 1997) and lipid peroxidation *in vivo* (Walker et al 1990), both of which may damage mitochondrial membrane lipids, (Ames et al 1995), deactivate mitochondrial ETC components and ATPase (Zhang et al 1990).

CyA causes renal and peripheral vasoconstriction inducing nephrotoxicity and systemic hypertension (Myers et al 1988; L'Azou et al 1994; Bartholomeusz et al 1996). In CyA-induced nephrotoxicity, renal vasoconstriction causes a reduction of glomerular filtration rate, which stimulates antidiuretic hormone (ADH) release and production of renin and angiotensin II (Barros et al 1987). The latter two vasoactive substances can induce further peripheral vasoconstriction, therefore, resulting in further impairment of glomerular function and aggravating hypertension (Siegel et al 1983). ADH enhances re-adsorption of H_2O in the renal tubules increasing blood volume, which also aggravates high blood pressure. Long-term vasoconstriction reduced capillary collateral circulation and finally results in a reduction of capillary network in muscle. It has been demonstrated that patients with systemic hypertension had a capillary rarefaction in skeletal muscle (Henrich et al 1988). Biring et al (1998) also found that CyA *in vivo* significantly reduced capillary-to fibre ratio and capillary density in rat extensor digitorum longus (EDL) muscle type I and type IIb fibres. The reduced capillarity in skeletal muscle may increase peripheral resistance, aggravating hypertension. With a reduction in muscle capillarity, the muscle oxidative capacity may also decline due to limitation to oxygen supply. Consequently, muscle energy metabolism may be reduced in skeletal muscle of hypertensive patients. Reduction of resting muscle PCr concentration and PCr regeneration during post exercise recovery have been reported in a group of hypertensive patients (Ronquist et al 1995). A low proportion of type I fibres (35% in patients with

hypertension vs. 40% in controls, $P < 0.05$) in skeletal muscle were found in the hypertensive patients (Juhlin-Dannfelt et al 1979). Once again, low proportion of type I fibres indicated the muscle oxidative capacity would be reduced since type I fibres have high oxidative potential (see Section 2.3.3). It is possible, therefore, that CyA induced hypertension contribute to the limitation of mitochondrial function in skeletal muscle. In the present study, 5 LTx recipients had mild hypertension even with medication. Unfortunately, the muscle capillary in these patients was not examined. Consequently, the present study can not proved any evidence related to muscle capillarity post-lung transplantation.

4.4.7. Anaemia

Finally, reduced exercise performance has been related to acute and chronic anaemia (Woodson 1978). In this study the average haemoglobin count of patients was 115.5 g L^{-1}, which is just in a low level of normal region. Consequently, deficiencies in physical performance and mitochondrial injury in the LTx group are unlikely to be linked to anaemia.

4.5. Conclusion

In conclusion, this study has demonstrated that LTx recipients have a low exercise capacity, characterised by shorter exercise duration at they ceased exercise at a lower workrate during incremental exercise and a lower $\dot{V}O_2$ peak, and lower LT during exercise compared with controls. Comparing with the controls, the resting skeletal muscles in LTx patients exhibited: (1) A lower proportions of muscle type I fibre. (2) Abnormal metabolism in resting muscle including a lower ATP concentration associated with higher levels of lactate and IMP. (3) Lower mitochondrial enzyme activities and higher PFK activity. (4) Low MAPR with various substrates. Low activity of mitochondrial enzymes was consistent with the impaired function

of mitochondrial ATP production. The metabolic alterations in LTx may be a result of low MAPR and a low proportion of type I muscle fibres. The abnormal alterations of skeletal muscle in LTx most likely occurred preoperatively and persist after transplantation. In terms of the causes, cardiac-pulmonary disease induced chronic hypoxemia and/or physical muscle disuse may be the cause of peripheral skeletal muscle deconditioning. In addition, CyA, perhaps PNL and AZA may impair mitochondrial capacity in LTx patients. Although there are many potential causes of exercise limitation in LTx recipients, impaired oxidative capacity of the peripheral skeletal muscle shown in this study may prove the most significant and the most prevalent.

CHAPTER 5. THE EFFECT OF CYCLOSPORINE ON *IN VITRO* MITOCHONDRIAL ATP PRODUCTION RATE IN RAT SKELETAL MUSCLE

5.1. INTRODUCTION

Cyclosporine A (CyA) (Sandimmune ^{TM}) is a cyclic polypeptide consisting of 11 amino acids and is a metabolite of the fungus species *Tolypocladium Inflatum Gams* (Dreyfuss et al 1976). The CyA immunosuppressive effect is the result of CyA binding to intracellular receptors (cyclophilins) and decreasing T-lymphocyte function (Cyert 1992). CyA is a potent immunosuppressive agent and has been widely used in organ transplantation. In combination with prednisone and azathioprine (triple-drug immunotherapy) CyA significantly decreases the frequency of infections and graft rejection (Andreone et al 1986). CyA also improved the survival rate of organ tranplantation (Oyer et al 1983; Fragomeni and Kaye 1988) and has been regarded as a major contributor to the improved survival of patients with organ transplantation (Fragomeni and Kaye 1988). However, CyA has a number of side effects. The most common and severe of these are nephrotoxicity (Salaman 1984; Bennett et al 1994) and systemic hypertension (Morrison et al 1993; Textor et al 1994). There are a few reports of CyA-induced skeletal muscle myopathy (Goy et al 1989; Fernandez-Sola et al 1990).

Previous studies have shown that CyA can inhibit the O_2 consumption of isolated kidney cortical mitochondria in rats (Jung and Pergande 1985; Simon et al 1997) and humans (Jung and Reinholdt 1987) in a dose-dependent manner. It has also been demonstrated that CyA significantly depresses mitochondrial function in the liver (Broekemeijer et al 1989; Samuta et al 1993), cardiac myocytes (Altschuld 1992) and in rat skeletal muscle (Hokanson et al 1995; Mercier et al 1995). The latter observations are most relevant to the present study. The

CyA concentration employed by Hokanson et al (1995) and Mercier et al (1995), however, was much higher than the level of CyA which is required (150 to 400 ng ml^{-1}) in whole blood to provide adequate immunosuppression in the early postoperative phase as suggested by Baumgartner et al (1987). It is not clear, therefore, that CyA will inhibit skeletal muscle mitochondria at a concentration in the clinical range.

It is possible that the low MAPR observed in LTx recipients is due to the effect of CyA. No studies, however, have examined the effects of CyA on MAPR in skeletal muscle. Accordingly, the purpose of this study is to examine the effects of high and low CyA concentrations on MAPR of skeletal muscle in rats. High CyA concentrations were 50 μg ml^{-1} and 25 μg ml^{-1}. The latter was chosen as Hokanson et al (1995) utilised this concentration in their study of the effect of CyA on mitochondrial O_2 consumption. Choosing a low concentration of CsA (1 μg ml^{-1}) was based on clinical considerations. Post-operatively, most patients with organ transplantation take maintenance dosages of CyA. In these patients blood CyA concentrations were usually maintained at 150-400 ng ml^{-1} during the first 6 months (Keown et al 1982; Baumgartner et al 1987; Kahan 1987). As indicated in the previous Chapter, the average whole blood CyA concentration in the LTx patients was 266 ± 30 ng ml^{-1} (from 198 ng ml^{-1} to 412 ng ml^{-1}). There is, however, limited data available for skeletal muscle CyA concentration in patients on maintenance dosages of CyA. Lensmeyer et al (1988) have reported that deposits of CyA and a CyA metabolite (M17 hydroxycyclosporine) were found in the skeletal muscle of three of the patients post-mortem. This group included a heart transplant recipient, a bone marrow transplant recipient and a liver transplant recipient. The heart transplant recipient was treated with CyA for 3 days (CyA concentration not given), but received 82 mg of CyA intravenous (iv) one day before death. The bone marrow transplant recipient took a maintenance dose of CyA for 3 months with a final dose of 50gm via intravenous injection 3 days before death. The liver transplantation patient received CyA for 3 months with 900 mg of CyA (oral) on the day of death. This study showed that the

muscle concentration of the CyA metabolite, M17, in the three patients was ~2-3 fold higher than the blood level indicating that the sum of CyA and metabolites was greater in skeletal muscle than the blood. Dowling et al (1990) provided further evidence that CyA concentrations are higher in muscle than blood. They examined the distribution of CyA one hour after giving aerosolised CyA to canine lung transplant recipients. The whole blood CyA level was 185 ± 116 ng ml^{-1} and in two separate measures, skeletal muscle CyA levels were 414 and 435 ng ml^{-1}. Wagner et al (1987) observed the tissue distribution and metabolism of CyA in rats. They administered single-dose ^{3}H-cyclosporine with an oral dose of 10 mg kg^{-1} or intravenous dose 3 mg kg^{-1} to rats. The ratio of muscle ^{3}H-cyclosporine and blood radioactivity (muscle/blood) was 7 and 6.7 after 24 hours oral and 8 hours intravenous administration of the drug, respectively. They also administered multiple oral doses of ^{3}H-cyclosporine at 10 mg kg^{-1} per day to rats for 21 days. After 21 days, the rats were sacrificed and the ^{3}H-cyclosporine radioactivity was 5-fold and 8-fold higher in muscle compared with the ^{3}H-cyclosporine radioactivity in blood at 8 hours and 24 hours, respectively, after the last dose of drug. The administration of multiple doses CyA mimics the situation of LTx patients taking maintain does CyA after transplantation. It is likely, therefore, that in patients undertaking CyA therapy, the concentration of CyA in the muscle would be at least 1 μg ml^{-1} if the blood CyA level is at 300-400 ng ml^{-1}.

This study will investigate the effects of CyA on *in vitro* MAPR in skeletal muscle of rats. Especially low concentration (1 μg ml^{-1}) CyA have not been previously examined for MAPR in skeletal muscle. The low concentration CyA may be close to the muscle CyA concentration in skeletal muscle in clinical situations. The hypotheses in the present study is: CyA may significantly inhibit *in vitro* MAPR in presence of various substrate combinations in both high concentration CyA (50 & 25 μg ml^{-1}) and low concentration (1 μg ml^{-1}).

5.2. METHODS

5.2.1. Animals and Skeletal Muscle Sampling

5.2.1.1. Animals

Eight female Sprague-Dawley rats, aged 57 ± 4.5 days (mean ± SEM) and weighing 200 ± 7.9 g (mean ± SEM) were sacrificed for this study.

5.2.1.2. Muscle sampling

The animals were euthanased with an overdose of halothane anaesthetic. The red gastrocnemius muscles were excised (~125-230 mg) to determine the MAPR. The muscle management, homogenisation and isolation of mitochondria were performed using the same techniques as those used in the human studies, except that mitochondrial suspensions were a 1:500 dilution for the final MAPR analyses. The average values of muscle weight, isolated mitochondrial protein concentration and the final mitochondrial protein in each cuvette are shown in Table 5.1.

Table 5.1. Muscle Weight and Mitochondrial Protein Contents of Rats

Mitochondrial Preparation	Average Values
Muscle weight (mg)	148.2 ± 11.9
Protein concentration in mitochondrial suspension (mg ml^{-1})	3.44 ± 0.43
Mitochondrial suspension protein content in each cuvette (ng ml^{-1}).	68.1 ± 8.5

Values are mean ± SEM (*n* = *8*).

5.2.2. Cyclosporine A Preparation

Cyclosporine A, which was kindly provided by Sandoz Australia, was dissolved in 50% ethanol at room temperature. Stock solutions of CyA (10 mg ml^{-1}, 5 mg ml^{-1} and 0.2 mg ml^{-1}) were prepared. A 50% ethanol solution was used as blank. To each cuvette, 0.005 ml of blank or a stock solution of CyA was added to produce a final concentration of 50 μg ml^{-1}, 25 μg ml^{-1}, and 1 μg ml^{-1} of CyA. The blank (0 μg ml^{-1}) was used to add an equal volume of 50% ethanol as in the other two testing situations. The content of ADP and volume of mitochondrial suspension added to each cuvette was the same as in the previous study (Chapter 4). Hence MAPR was examined in the presence of 50 μg ml^{-1}, 25 μg ml^{-1}, and 1 μg ml^{-1} and 0 μg ml^{-1} of CyA.

5.2.3. MAPR Measurement

This study employed the same technique used to measure the MAPR in the human studies. Before adding ADP the isolated mitochondria were incubated with CyA at three different concentrations CyA (50 μg ml^{-1}, 25 μg ml^{-1}, and 1 μg ml^{-1}) and 50% ethanol (CyA 0 μg ml^{-1}) for 5 minutes at 25 °C. After adding ADP, MAPR with each substrate combination at different CyA concentrations was measured at same time. MAPR is expressed as mmol min^{-1} g^{-1} protein of the mitochondrial suspension. The combinations of substrates or substrate and inhibitor used in this study were P + M, PC + M, PPKM and S + R. The methods were as described in Chapter 3.

5.2.4. Statistical Analyses

A two-factor analysis of variance (ANOVA) (Microsoft Excel statistical package) was used to

analyse data of MAPR from the three different concentrations of CyA and the blank. When a significant F-value was obtained Newman-Kuels post-hoc analysis was employed to determine the differences. A level of $P < 0.05$ was accepted as a significant difference. The data are presented as mean ± standard error of the mean (SEM).

5.3. RESULTS

The effects of different concentrations of CyA on the MAPR of rat skeletal muscle are shown in Table 5.2 and Figure 5.1. MAPR in the presence of the four combinations of substrates or substrates and inhibitor (S + R) was depressed by CyA at a concentration of 1 μg ml^{-1} compared with the blank ($P < 0.01$). The MAPR in the presence of a CyA concentration of 1 μg ml^{-1} was reduced by 10% to 12% compared with the blank. Similarly, MAPR in each circumstance was also lower in the presence of CyA at a concentration of 50 μg ml^{-1} and 25 μg ml^{-1} compared with a CyA concentration of 1 μg ml^{-1} and blank ($P < 0.05$ & $P < 0.01$, respectively). The levels of MAPR with various substrates were very similar between the 50 μg ml^{-1} and 25 μg ml^{-1} CyA groups. MAPR in the prescence of 25 μg ml^{-1} of CyA was reduced by 21% to 25% compared with the blank. There were no differences when comparing the reduction of MAPR with various substrate combinations and the same CyA concentration ($P > 0.05$). The decline of MAPR in the presence of various substrates is shown in Table 5.3.

Table 5.2. Effect of Cyclosporine A on MAPR

Substrates	0 μg ml^{-1}	1 μg ml^{-1}	25 μg ml^{-1}	50 μg ml^{-1}
PPKM	0.69 ± 0.07	0.60 ±0.05*	0.54 ± 0.04*†	0.55 ± 0.04*†
P + M	0.57 ± 0.07	0.51 ±0.08*	0.45 ± 0.05*†	0.42 ± 0.06*†
PC + M	0.36 ± 0.03	0.32 ±0.02*	0.28 ± 0.01*†	0.29 ± 0.01*†
S + R	0.29 ± 0.05	0.25 ±0.04*	0.21 ± 0.03*†	0.21 ± 0.03*†

Values are mean ± SEM, expressed in mmol min^{-1} g^{-1} protein of mitochondrial suspension (n = 8).* $P < 0.01$ compared with 0 μg ml^{-1}. †$P < 0.01$ compared with 1 μg ml^{-1}.

Table 5.3. Percentage Reduction in MAPR at Different Concentrations of CyA

Substrates	1:0 μg ml^{-1}	25:1 μg ml^{-1}	25: 0 μg ml^{-1}	50:0 μg ml^{-1}
PPKM	12 ± 3	10 ± 4	21 ± 4	18 ± 5
P + M	11 ± 3	10 ± 3	20 ± 5	25 ± 6
PC + M	10 ± 3	11 ± 4	20 ± 4	19 ± 6
S + R	12 ± 2	14 ± 2	25 ± 3	28 ± 4

Values are mean ± SEM, expressed in percentage [(a-b)/b%] %. n = 8.

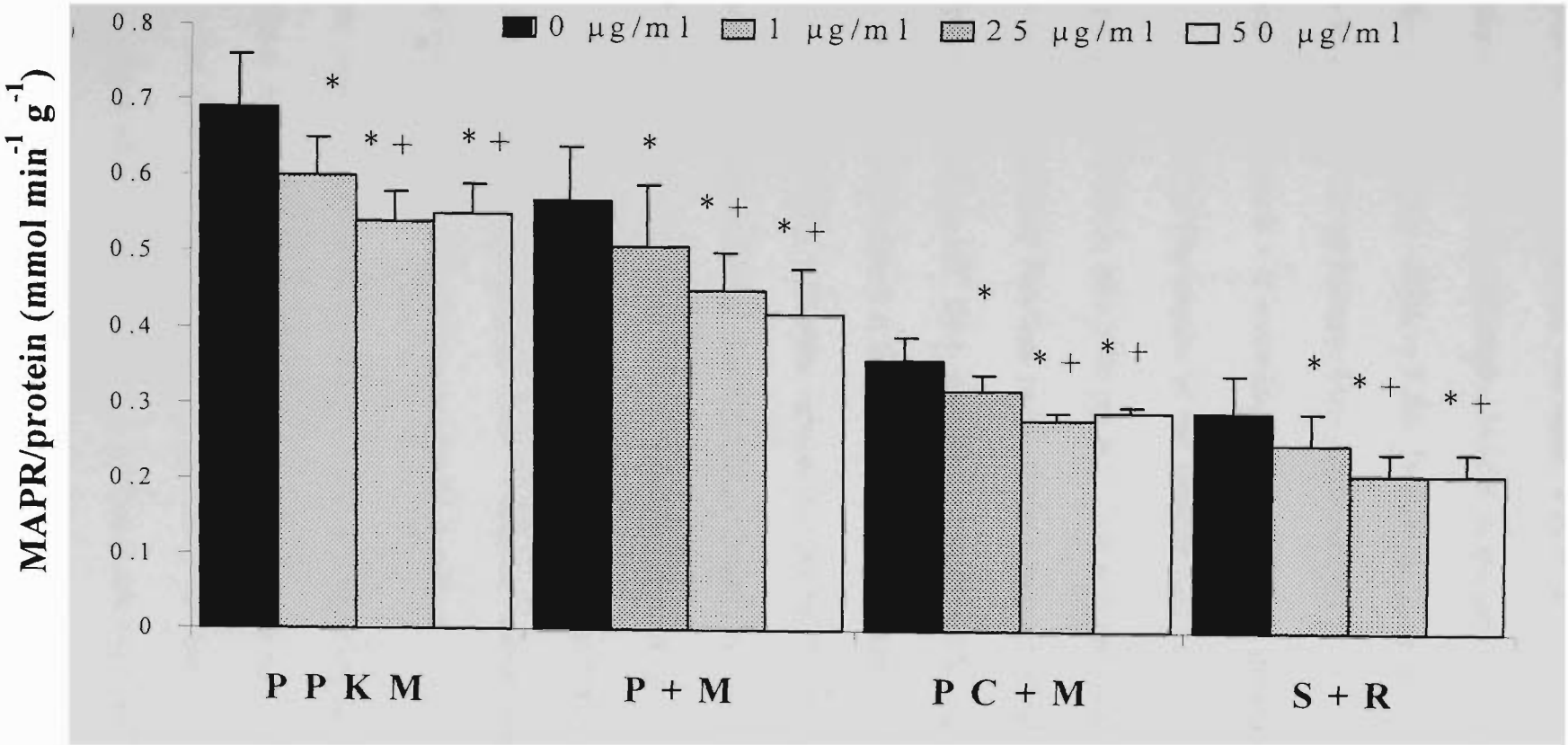


Figure 5.1. Effect of CyA on MAPR

Values are mean ± SEM, expressed in mmol. $min^{-1}g^{-1}$ protein. ($n = 8$). * $P < 0.05$ and $^{\dagger}P < 0.01$ compared with 0 µg ml^{-1}. $^{*}P < 0.05$, and $^{+}P < 0.01$ compared with 1 µg ml^{-1}.

5.4. DISCUSSION

The present study demonstrates that CyA inhibits *in vitro* MAPR in rat skeletal muscle mitochondria. This observation was made with both low (1 μg ml^{-1}) and high (25 μg ml^{-1}) concentrations of CyA. Significantly, MAPR in the presence of 1 μg ml^{-1} CyA concentration at a level of declined by ~10% to 12%. This result is similar in magnitude to the findings of Simon et al (1997) in rat kidney. They reported that RCR of rat kidney mitochondria using substrate and inhibitor S + R was reduced by 10% in the presence of 1 μM CyA (1 μmmol L^{-1} CyA ~ 1.03 μg ml^{-1}). The results of the present study are also consistent with the study of Hokanson et al (1995), in which 25 μg ml^{-1} CyA concentration significantly reduced isolated mitochondrial respiratory function in the skeletal muscle of rats. In the present study MAPR in the presence of 25 μg ml^{-1} CyA was reduced by 20 ± 5% with P + M and 25 ± 3% with S + R. By comparison, Hokanson et al (1995) found that State 3 mitochondrial respiration in the presence of P + M and S + R were reduced by the 34.7% and 18.3%, respectively. Jung and Pergande (1985) demonstrated that CyA significantly reduced the rat kidney mitochondrial State 3 respiration in a dose-dependent manner over a range of CyA concentrations from 10 μg ml^{-1} to 75 μg ml^{-1}, but CyA at 5 and 1 μg ml^{-1} concentration did not affect mitochondrial respiration. The data in the present study is also consistent with a dose-dependent affect on mitochondrial function in skeletal muscle mitochondria.

It is clear from the present study that the CyA concentration (1μg ml^{-1}) likely to be present in the skeletal muscles of LTx recipients potentially reduces mitochondrial function. In the previous the MAPR with various substrates in terms of mitochondrial suspension protein was lower by about ~16 to 45% in the LTx patients compared with the controls. In the present study, 1μg ml^{-1} CyA, however, only depressed MAPR by ~10 to 12%. The high CyA concentrations (50 μg ml^{-1} and 25 μg ml^{-1}) used are not implicated in a clinical situation, because the skeletal muscle CyA concentration may never reach to 50 μg ml^{-1} and 25 μg ml^{-1}

in the heart and lung transplantation patients who undertake CyA. Therefore, the results in this study strongly suggest that CyA is a factor, but not the only factor, which contributes to the reduction of MAPR and exercise limitation in the LTx patients.

The cause of CyA-toxicity on mitochondria is still unclear. A number of explanations have been suggested previously. CyA may directly inhibit one or all enzyme complexes in the ETC. Lemmi et al (1990) demonstrated that CyA inhibited *in vitro* Complex II of rat renal cortical mitochondria. In the present study, however, this does not appear to be the case as CyA depressed MAPR to similar levels using combinations of various substrates. As previously indicated, succinate enters the ETC via Complex II and rotenone inhibits electron transfer from Complex I to UQ. Hence, if CyA reduced the activity of Complex I alone, then MAPR in the presence of S + R would have been unaffected. As described in Section 2.2.3.3, most electrons carried by $FADH_2$ enter Complex III directly from fatty acid β-oxidation directly to UQ by electron-transferring flavoprotein (ETFP). Hence, if CyA reduced the activity of Complex II and I alone, MAPR in the presence of PC + M would have reduced to a less extent than that observed with P + M and S + R as substrates. Therefore, these results indicate that CyA did not specifically effect Complex I, II or III. Hokanson et al (1995) have pointed out that inhibition of mitochondrial respiration by CyA is possibly due to the effects of CyA on mitochondrial membrane, rather than its direct affect on the specific protein complex in the ECT of mitochondria. They also suggested that CyA might affect the electron flux through the ETC in muscle mitochondria. The data presented here, however, does not negate the possibility that CyA could have a deleterious affect on other protein complexes or components in the ETC, such as Complex IV, ATP synthase and other enzymes.

It is also possible that the inhibitory effect of CyA on mitochondrial function could be due to changes in permeability of Ca^{2+}in the mitochondrial membrane. Cyclosporine A can block MPTP to decrease Ca^{2+} efflux from the matrix of mitochondrion into the cytosol and this may result in an accumulation of excess Ca^{2+} in the mitochondrial matrix (Altschuld et al 1992;

Richter et al 1990). Mitochondrial Ca^{2+} overload has been linked to mitochondrial damage, both morphologically and functionally (Lochner et al 1987, Darley-Usmar et al 1990). Salducci et al (1996) used CyA to produce a Ca^{2+} overload in liver mitochondria. This process resulted in impairment of respiration in the mitochondria. The authors then utilised trimetazidine (TMZ), an antianginal drug, to increase Ca^{2+} release from mitochondria, which reversed the detrimental effects of CyA on mitochondrial respiration. Accordingly, Ca^{2+} overload in mitochondria can result in impairment of mitochondria function. Fournier et al (1987) and Crompton et al (1992) have suggested that decreased Ca^{2+} efflux may reduce the proton gradient across the inner mitochondrial membrane leading to a reduction in ATP synthesis. In support of this hypothesis, Zamzami et al (1995) have demonstrated that CyA reduces the electrochemical potential of the mitochondrial membrane. When the membrane potential drops, ROS could be generated. The over-production of ROS can impair mitochondrial function, which will be discussed subsequently.

As mentioned in Chapter 4, a number of studies have shown that CyA can protect mitochondria via blockage of the MPTP, reducing ischaemic-reperfusion damage on the cardiomyocyte mitochondria (Griffiths and Halestrap. 1993) and improving ATP recovery in hepatocytes after anoxic-reoxygenation (Qian et al 1997). The protective effect of CyA, however, is not clear. Elimadi et al (1997) found that CyA was only transiently protected the liver mitochondrial swelling induced by Ca^{2+} plus tert-butylhydroperoxide. After the initial protection, CyA increased the mitochondrial swelling. Griffiths and Halestrap. (1993) demonstrated that 0.2 μM CyA restored the ATP/ADP ratio and AMP contents to pre-ischaemic levels and after rat heart reperfusion. The protective effect, however, was reversed by 1 μM CyA. There is very limited information about MPTP in mitochondria of skeletal muscle. Recently, Fontaine et al (1998) found that the skeletal muscle mitochondria have the permeability transition pore. Similar to the pore in heart and liver mitochondria, Ca^{2+} can be accumulated in mitochondria via the pore and the pore can be blocked by cyclosporine A in

skeletal muscle.

The another possibility for CyA reduced mitochondrial function may involve production of the oxidants as previously described in section 2.3.5.2. It has been documented that CyA can induce oxidative stress in rat hepatocytes (Wolf et al 1997). This oxidative stress is characterised by overproduction of ROS, lipid peroxidation, loss of protein thiols and a decrease in the molar ratios of glutathione and glutathione disulfide (GSH/GSSG). In addition, the oxidative stress was also associated with an increase in the release of lactate dehydrogenases which indicates cell damage. Zhang et al (1990) demonstrated that ROS deactivates ETC complexes and inhibits the activity of oxidative enzymes. Brudvig et al (1980) and Cooper and Brown (1995) have also demonstrated that nitric oxide (NO) inhibits mitochondrial cytochrome oxidase. Peroxynitrite ($ONOO^{\bullet}$), which is produced from NO, can also reduce mitochondrial respiration at Complexes I, II, III (Cassina and Radi 1996) and possibly IV (Lizasoain et al 1996). Furthermore, CyA can induce *in vitro* lipid peroxidation in renal cortical mitochondria (Walker et al 1986) and increase *in vivo* lipid peroxidation in rat mitochondria (Walker et al 1990). Peroxidation of mitochondrial membrane lipids impairs the electrochemical potential of the organelle membrane and leads to depletion of ATP, finally causing irreversible cell injury (Carini et al 1992). Following impairment of mitochondrial membrane, proton leakage cross the mitochondrial inner membrane may increase and uncoupling the ATP production in ATP synthase.

Tsuchiya et al (1990) have demonstrated reported that halothane, a volatile anaesthetic, can impair isolated rat liver mitochondrial oxygen consumption (State 3 respiration) in the presence of α-ketoglutarate *in vitro*, but unaffected the mitochondrial function with succinate. Recently, Miro et al (1999) have also reported that halothane can decrease human skeletal muscle mitochondrial oxidation of glutamate, succinate and ascorbate. Although halothane was used for rats in the present study, the results of the present study still indicate the negative effects of CyA on MAPR *in vitro*. Because the sample used for the blank was the same

sample used for the three CyA groups. Consequently, if halothane affects MAPR in this study, all groups would be affected to the same extent. Nevertheless the combined effect of CyA and Halothane might result in an amplified inhibition attributed to CyA alone. Therefore, further study is required to determine the magnitude of inhibition produced by CyA alone.

5.5. CONCLUSION

In conclusion, this study has shown that CyA at 50 $\mu g\ ml^{-1}$, 25 $\mu g\ ml^{-1}$ and 1 $\mu g\ ml^{-1}$ concentrations significantly reduces the MAPR in the presence of various combinations of substrates including substrate and inhibitor (S + R). It is significant that CyA concentration is likely to exist in muscle of LTx patients (1 $\mu g\ ml^{-1}$) produced a decline in MAPR of 10 to 12%. This reduction, however, was less than observed difference in MAPR between the LTx patients and controls (16 to 45%). Therefore, these results indicated CyA could reduce mitochondrial function in skeletal muscle post lung transplantation contributing to limitation of exercise performance.

CHAPTER 6. MITOCHONDRIAL CAPACITY IN SKELETAL MUSCLE OF RESISTANCE-TRAINED SUBJECTS

6.1. INTRODUCTION

Resistance training is an effective exercise mode for enlargement of skeletal muscle mass, enhancement of muscle strength and force generation in healthy subjects (MacDougall et al 1977; Komi et al 1978; McDonagh and Davies 1984; Jones and Rutherford 1987; Alway et al 1992; Starkey et al 1996). Recently, it has been demonstrated that resistance training also effectively improves the ageing-induced muscle degeneration or muscle atrophy in elderly people (Fielding 1995; Sipilä and Suominen 1995; Skelton et al 1995; Evans 1997). This exercise mode has been adopted in many programs for improving muscle bulk and strength in aspects of sports medicine or medical rehabilitation. It has been demonstrated that resistance training improves the muscle weakness and muscle atrophy which result from muscle immobilisation and disuse (MacDougall et al 1977; 1980, Sale et al 1982; Ingemann-Hansen and Halkjaer-Kristensen 1985b). Patients with severe heart and lung disease typically experience long-term physical inactivity and bed rest combined with the pathological processes, such as heart failure or hypoxaemia, which may result in skeletal muscle deficiencies (see Section 2.3.4).

In the patients with severe heart and lung diseases, these muscle deficiencies are characterised by a low proportion of muscle type I fibres, muscle fibre atrophy, muscle metabolite abnormalities and low mitochondrial capacity including low mitochondrial volume and low oxidative enzyme activity in skeletal muscle (see Section 2.3.4). It has been demonstrated that the skeletal muscle deficiencies can be partially reversed by exercise training via both endurance training (Chati et al 1996; Hambrecht et al 1997) and resistance training (Gosselink and Decramer 1998). Previous studies have demonstrated that the major benefits of endurance

training are improving oxidative or mitochondrial capacity (Wibom et al 1992) and enhancing exercise endurance. Patients with severe cardiopulmonary disease, however, may be too weak to undertake aerobic exercise. Resistance training of some types, such as isotonic and circuit weight training, can be safely employed in these patients. Resistance training has been recommended that only involves a few repetitions per session (Feigenhaum and Pollock 1999) and this should have a low impact on the cardiovascular system. Verrill and Ribisl (1996) have reviewed previous studies and summarised the advantage and consequences of resistance training for cardiac rehabilitation. Resistance training has become a popular exercise mode in rehabilitation programs for patients with cardiopulmonary diseases in recent years (Verrill and Ribisl 1996). As previously indicated, the main effects of resistance training are increases in muscle bulk and improves muscle strength and force. Undertaking resistance training to increase skeletal muscle mass and improve muscle function, may allow patients with severe cardiopulmonary disease to perform the aerobic exercise training more adequately. It has been demonstrated that an exercise training program combining endurance training and resistance training can more effectively improve the exercise performance and oxidative capacity in healthy aged subjects (Ferketich et al 1998).

Skeletal muscle hypertrophy of both type II and I fibres is a well-accepted characteristic of muscle in resistance-trained subjects (Dons et al 1979; Lüthi et al 1986; Alway et al 1988; Jurimae et al 1996). However, the effects of resistance training on many other muscle characteristics remains controversial. For example, it has been demonstrated that the activity of glycolytic enzymes in resistance training is increased (Costill et al 1979), unchanged (Houston et al 1983), or even decreased (Tesch et al 1987). Similarly, the effect of resistance training on mitochondrial capacity in the hypertrophied muscle is also not clear. Previous studies have shown various results for skeletal muscle mitochondrial volume density as well as oxidative enzyme activity of mitochondria following resistance training or strength training (see Chapter 2.2.2.3). No studies, however, have investigated MAPR in skeletal muscle after

resistance training. In addition, the effect of resistance training on muscle metabolism is also unclear. It was reported that resistance training increased the levels of ATP, PCr and Cr in resting muscle (MacDougall et al 1977). However, the information about the effects of resistance training on muscle metabolites is limited.

Although resistance training (or strength training) increases muscle maximal force, reports of the effects on fatigue resistance with muscle hypertrophy, found in previous studies, are equivocal. There are reports that resistance training improves (Hickson et al 1980; 1988) or has no effect on endurance performance during exercise (Bishop and Jenkins 1996). In view of these findings, the hypotheses in the present study are:

(1). Resistance-trained subjects have a greater muscle bulk and higher muscle power output during one-legged exercise compared with controls.

(2). Resistance trained subjects have increased total muscle oxidative capacity indicated by MAPR and oxidative enzyme activities in skeletal muscle.

Therefore, this study was designed to examine:

(a). Muscle mass, power output and fatigue during intense exercise in resistance-trained subjects.

(b).The mitochondrial capacity in skeletal muscle of resistance-trained subjects.

(c). The effects of long-term resistance training on exercise endurance and metabolism in skeletal muscle during fatiguing exercise.

6.2. METHODS

6.2.1. Subjects

Sixteen volunteers participated in this study. They included 8 untrained male controls (UT) and 8 male resistance-trained (RT) subjects. The UT subjects did not regularly participate in sporting activities, but were recreationally active in some instances. The RT subjects were recruited through the university, local competitive clubs and representative teams and had been training continuously for at least two years. The RT subjects were selected on the basis of being able to lift at least 1 to 1½ times their body weight during squatting exercise with free weights. The RT group had typically trained 4-5 times per week at ~1 hour per session.

The height and body mass of subjects were measured using a stadiometer (Novel Inc, Addison Illinois, USA) and an electronic scale (Sauter E1200, West Germany) with a sensitivity of 0.005 kg, respectively. Subcutaneous skinfold thickness was measured at the following eight sites, triceps muscle, biceps muscle, subscapular, mid-axilla, suprailiac, abdominal, anterior thigh and medial calf using Harpenden calipers (British Indicators, W. Sussex, Great Britain). The sum of these of 8 sites average values (Sum of 8) was used to calculate body fat percentage (% BF) (Siri, 1956) from body density using equations, which are listed in Appendix II (Siri 1956; Jackson & Pollock 1978; Jackson et al 1980). Estimation of the subjects' body fat was also determined by underwater densitometry (Brozek et al 1963). Total thigh volume was determined by water displacement. Thigh volume was obtained by subtracting the volume of the lower leg (at the lateral and medial epicondyles of the femur) from the volume of the whole leg (up to the gluteal furrow in the horizontal plane). The results for all subject skinfolds and limb volume measurement equation is shown in Appendix II: Cross-sectional area (CSA) of the whole thigh muscle including quadriceps and hamstring muscles was determined by anthropometric techniques according to the method of Housh et al

(1995). The thigh CSA was calculated using the following equation:

Total thigh muscle CSA = (4.68 × mid-thigh circumference in cm) – (2.09 × anterior thigh skinfold in mm) – 80.99.

6.2.2. Exercise Tests

All subjects performed separate tests to measure maximal aerobic power, as well as muscle function and fatigue in the knee extensor muscle of one leg.

6.2.2.1. Measurement of Maximal Aerobic Power

When the subjects arrived at the laboratory on the test day, they were weighed and had a catheter inserted into a superficial vein in the dorsum of the right hand for arterialised venous blood samples (see Section 3.4.1). Maximal aerobic power was determined through an incremental exercise test (25 watts per min) on an electrically braked cycle ergometer (Lode N. V. Groningen, Netherlands). The measurements and protocol have been described in Section 4.2.2.

6.2.2.2. Assessment of Muscle Function

Subjects were also weighed when they arrived at the laboratory for this test, but no invasive measures were performed. Maximal muscle strength and fatigability of the knee extensor muscle group was determined on an isokinetic dynamometer (Biodex Medical System, Shirley, New York, USA). Briefly, after a 3-minute warm-up on a cycle ergometer at 50 watts (W), subjects were strapped to the Biodex chair using belts across the hip, knee, ankle and chest, to restrict upper body movement. The knee extension repetitions were then performed in two separate tests to determine muscle function of the knee extensors. These tests were

measuring muscle torque-velocity relationship and leg muscle fatigability.

Muscle Torque-Velocity Relationship.

The first test measured peak muscle torque in the right leg only at a series of limb velocities. Subjects performed 2 practice maximal repetitions, followed by one-minute rest. Then 3 maximal repetitions at each velocity of 0, 60, 120, 180, 240, 300 and 360 degrees per second ($^{\circ}$ s^{-1}), each separated by a 2 minute rest. Data from these tests was used to build a torque-velocity relationship of the right knee extensor muscle group. Peak torque was expressed in Newton-meters (Nm).

Biodex Muscle Fatigue Test

After a recovery period of 30 minutes, the second test was performed on Biodex dynamometer. Subjects completed a standard test designed to rapidly induce muscular fatigue of the knee extensor muscles. This involved 50 repeated maximal knee extensions (180°s, 0.5Hz), modified from the procedure described by Thorstensson et al (1976a). The peak torque during the test was calculated as the mean of the 5 strongest of the first 10 contractions. The final torque during the test was calculated as the mean of the 5 weakest in the final 10 contractions. The total work was summed from the values of 50 repeated knee extensions. The fatigue index, expressed in terms of a percentage, was calculated using the following equation:

Fatigue Index (FI) % = [(peak torque-final torque)/peak torque] × 100.

Cybex Muscle Fatigue Test with Invasive Measures

The second muscle fatigue test was performed with invasive measurements using an isokinetic dynamometer (Cybex II Lumex Inc. Ronkoukowany, New York USA). Separate ergometers were used for practical reasons. A Biodex dynamometer was preferred for characterisation of muscle function of the different subject groups, but was located at a different campus to the Exercise Physiology Laboratory, where the invasive testing took place. Therefore, a Cybex dynamometer was used for the invasive muscle fatigue test, with

collection of muscle biopsies and blood samples, in the Exercise Physiology Laboratory, Footscray campus, Victoria University of Technology.

Before this test, subjects were also weighed and catheterised with a venous line for arterialised blood samples (see 3.2.1). Subjects were strapped to the Cybex chair, using seat belts to restrict upper body movement, and required to perform 50 repeated maximal knee extensions (180° 0.5Hz). Muscle torque in Newton-meters was determined for each knee extension. A fatigue index was also determined in this test by the manner described above. Muscle biopsies were taken at rest and immediately after exercise. Blood samples were collected at rest, at 25 and 50 knee repetitions during the fatigue test as well as at 1, 2, 5, 10, 20 and 30 minutes after exercise test for blood gas and plasma lactate analyses. The test protocol is shown in Figure 6.1.

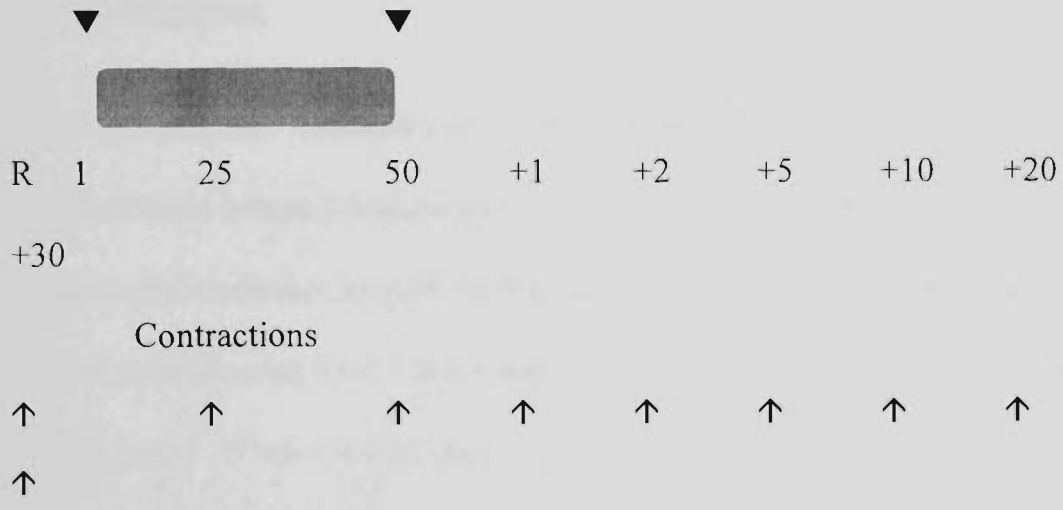


Figure 6.1. The protocols of Knee Extensions Fatigue Test

Blood and muscle sampling during the fatigue test: R, rest. ▬ 50 maximal knee extensions on Cybex isokinetic dynamometer (180°s, 0.5Hz). ↑ denotes blood sampling and ECG recording; ▼denotes muscle sampling.

6.2.3. Blood analyses

The techniques for analyses of blood gases and plasma lactate have been described in Section 3.4.2.

6.2.4. Muscle Biopsy and Muscle Analyses

Muscle samples were obtained from the vastus lateralis muscle, at rest and immediately after the 50 knee extensions. The procedure and techniques of biopsy and muscle samples management have been described in Section 3.5.1. The muscle samples were analysed for muscle fibre type proportion, activity of metabolic enzymes, resting and fatigue muscle metabolism and MAPR. A insufficiency of muscle sample prevented MAPR measurement in one resistance-trained subject and the determination of the enzymes KGDH, HAD, PFK HK, PK and PHOSPH activities in another RT subject. Likewise, KGDH and HAD were not measured in one untrained subject. The methods for muscle analyses have been described in Section 3.5.2.

6.2.5. Statistical Analyses

All data are presented as mean ± standard error of the mean (SEM). Muscle peak torque at different velocities, plasma lactate concentration during maximal aerobic tests and fatiguing tests using the Cybex dynamometer, as well as metabolites between the two groups and within each group, were compared using a two-factor analysis of variance (ANOVA) with repeated-measures (SPSS package). When a significant F-ratio was obtained, a Newman-Kuels post-hoc analysis was used to compare the variances between the two groups or between resting and fatigue muscles within each group. The data of MAPR, enzyme activities, lactate threshold during exercise between the two groups were compared by two-tailed Student's *t-test* (Microsoft Excel software package). A P value < 0.05 was considered to indicate a significant difference. Linear regressions were determined from the pooled data of the two groups and calculated using Microsoft Excel software package.

6.3. RESULTS

6.3.1. Anthropometric Measurement

Subjects' characteristics including those obtained using anthropometric measurement, are shown in Table 6.1. The thigh muscle cross-sectional area (CSA) in the RT group was higher than the UT group ($P < 0.01$) (Figure 6.2). The average values for the sum of 8 skinfolds and body fat calculated from skinfolds (%BF) were not different between the two groups. The leg thigh volume of the two groups were similar, but the anterior thigh skinfold was less in the RT subjects compared with the UT ($P < 0.05$). The results indicate that RT had less fat and larger muscle mass in the thigh. The UT subjects were slightly taller than the RT subjects ($P < 0.05$). There were no differences in other variables between the RT and UT groups.

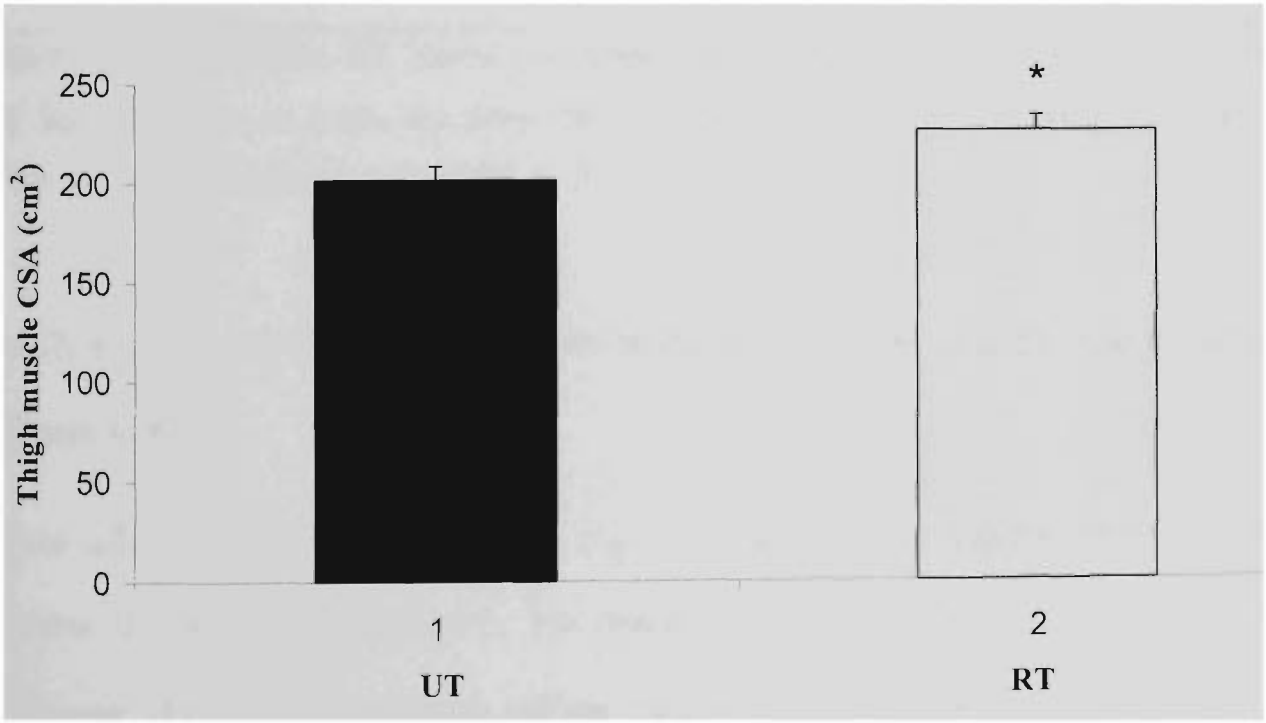


Figure 6.2. Cross-Sectional Area of Thigh Muscle

RT, resistance-trained subjects ($n = 8$); UT, untrained subjects ($n = 8$); mean ± SEM; ** $P < 0.01$.

Table 6.1. Subjects Characteristics and Results of Anthropometric Measurements

	UT	RT
Male	8	8
Age (year)	26.4 ± 1.4	25.6 ± 2.9
Height (cm)	183 ± 2.	176 ± 2*
Body Mass (kg)	80.4 ± 2.4	83.9 ± 2.4
Skinfolds [Sum of 8 (mm)]	99.4 ± 10.4	82.3 ± 7.5
Anterior thigh skinfold (mm)	15.0 ± 1.1	11.0 ± 1.2*
BF % (Sum of 8)	13.9 ± 1.5	11.5 ± 1.0
BF % (UWW)	17.5 ± 1.5	15.8 ± 1.8
Thigh volume (L)	5.78 ± 0.32	5.6 ± 0.18
C.S.A. of thigh muscle (cm^2)	201.8 ± 7.0	233.5 ± 7.4**

Values are mean ± SEM. RT, resistance-trained subjects (n = 8); UT, untrained subjects (n = 8); Sum of 8, sum of 8 site skinfolds; BF, body fat; UWW, underwater weigh % body fat; CSA, cross-sectional area. * $P < 0.05$. ** $P < 0.01$.

6.3.2. Pulmonary Function, Incremental Exercise Test and Muscle Function Assessment

There were no differences in pulmonary function, including vital capacity (VC) and FEV_1 between the two groups (Table 6.2). The results of the incremental exercise test, including $\dot{V}O_2$ peak, HRpeak, peak workrate and the duration of the test also showed no differences between the RT and the UT groups (Table 6.2). The value of LT, obtained during the measurement of maximal aerobic power, did not differ between RT and UT groups whether expressed in terms of absolute workrate or $\dot{V}O_2$ (Table 6.2).

In the Biodex dynamometer test, the leg muscle peak torque, at angular velocities of 60 through to 360° s^{-1}, were 12% to 22% higher in the RT than the UT group ($P < 0.05$, Table 6.3). The torque-velocity relationship is shown in Figure 6.3. Total work during the knee repetitions was higher in RT than UT ($P < 0.01$, Table 6.4). The peak work and the fatigue index (FI) in Biodex dynamometer fatiguing test were not different between the two groups (Table 6.4). The leg muscle peak torque, at angular velocities of 60 to 360° s^{-1} and total work had positive linear regressions with the CSA ($0.55 < r < 0.72$; $P < 0.05$). The regression of total work and CSA is shown in Figure 6.4.

The fatigue tests conducted on the Cybex dynamometer also showed that both the peak torque and FI did not differ between RT and UT (Table 6.4). The plasma lactate concentrations were not different in the RT group compared with the UT during the intense leg exercise and in recovery (Cybex test, Figure 6.5).

Table 6.2. Pulmonary Function and Incremental Exercise Test Results

	UT	RT
FEV_1 (L)	4.35 ± 0.18	4.42 ± 0.34
VC (L)	5.35 ± 0.28	5.35 ± 0.41
$\dot{V}O_2$ peak (ml $kg^{-1}min^{-1}$)	44.4 ± 1.8	44.6 ± 3.2
HRpeak (bpm)	190 ± 3	185 ± 3
Peak workrate (W)	325 ± 8	334 ± 16
Duration of exercise (min)	13.0 ± 0.3	13.4 ± 0.7
LT (Workrate, W)	101 ± 10	139 ± 21
LT ($\dot{V}O_2$, ml min^{-1} kg^{-1})	19 ±1.5	20 ± 3.2

Values are mean ± SEM. RT, resistance-trained subjects (n = 8); UT, untrained subjects (n = 8); FEV_1, forced expiratory volume in one second; VC, vital capacity; LT, lactate threshold; W, watts. * $P < 0.05$.

Table 6.3. Muscle Peak Torque (Nm) in Biodex Isokinetic Dynamometer Test Results

Angular velocity (°s)	UT	RT	% difference
0	197.0 ± 8.6	210.8 ± 9.1	+7
60	193.0 ± 6.7	216.9 ± 8.6*	+12
120	159.7 ± 4.1	186.3 ± 8.1*	+17
180	134.6 ± 3.7	159 ± 8.6*	+18
240	115.9 ± 4.1	139.6 ± 7.5*	+20
300	106.8 ± 4.8	127.1 ± 7.9*	+19
360	93.5 ± 4.2	114.0 ± 6.3*	+22

Values are mean ± SEM. RT, resistance-trained subjects (n = 8); UT, untrained controls (n = 8); % difference, (RT-UT)/UT%. * $P < 0.05$.

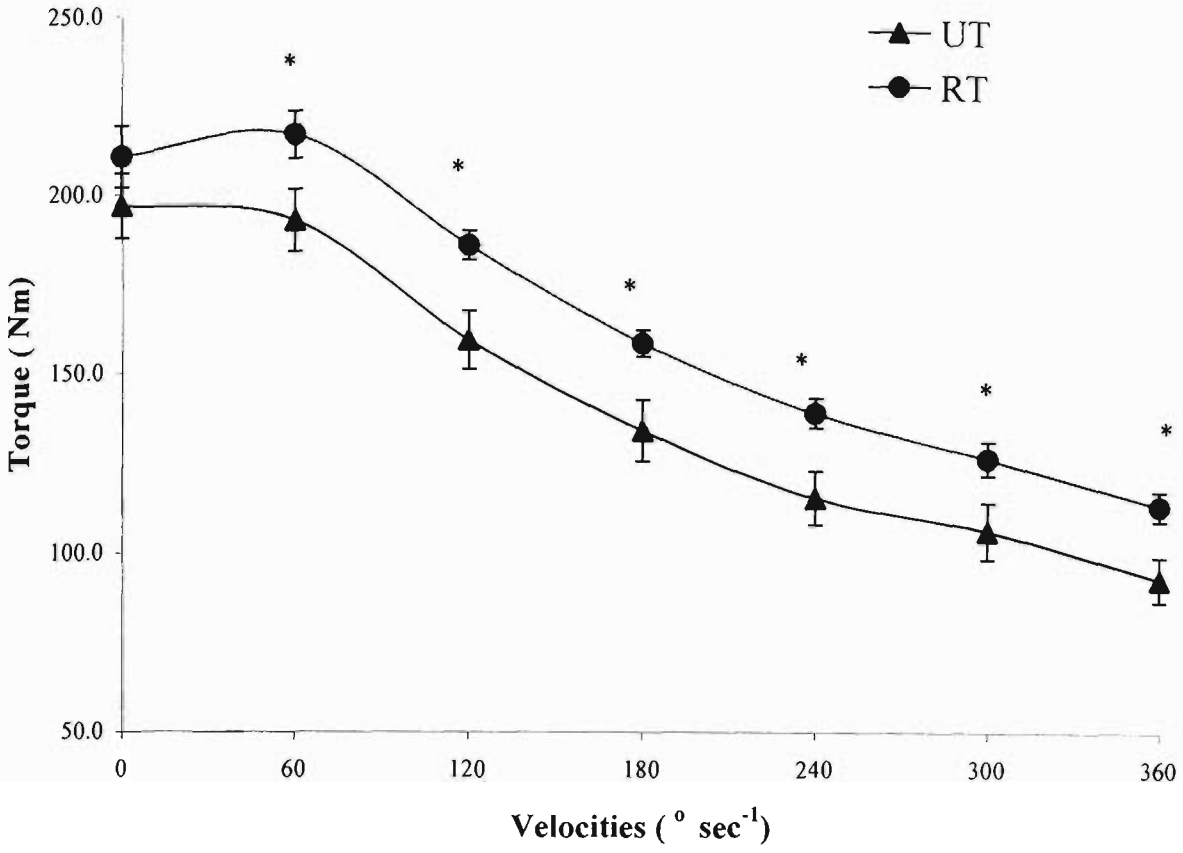


Figure 6.3. Torque-Velocity Relationship

● - Resistance-trained subjects (n = 8); ▲ - Untrained controls (n = 8); Mean ± SEM;

* $P < 0.05$.

Table 6.4. Fatigue Test Results in Biodex and Cybex Isokinetic Dynamometers

	UT	RT
Biodex tests		
Peak work (J)	143 ± 7.1	157 ± 5.4
Peak power (W)	164 ± 16	178 ± 18
FI_{Work} (%)	53.7 ± 5.0	46.4 ± 3.5
FI_{Power} (%)	51.2 ± 3.8	47.3 ± 2.7
Total work (J)	4992 ± 93	6058 ± 301*
Cybex tests		
Peak torque (Nm)	120 ± 3.5	119 ± 6.6
FI (%)	46.9 ± 3.2	42.3 ± 5.7

Values are mean ± SEM. RT, resistance-trained subjects (n = 8); UT, untrained controls (n = 8); W, watts; J, joules; *$P < 0.01$.

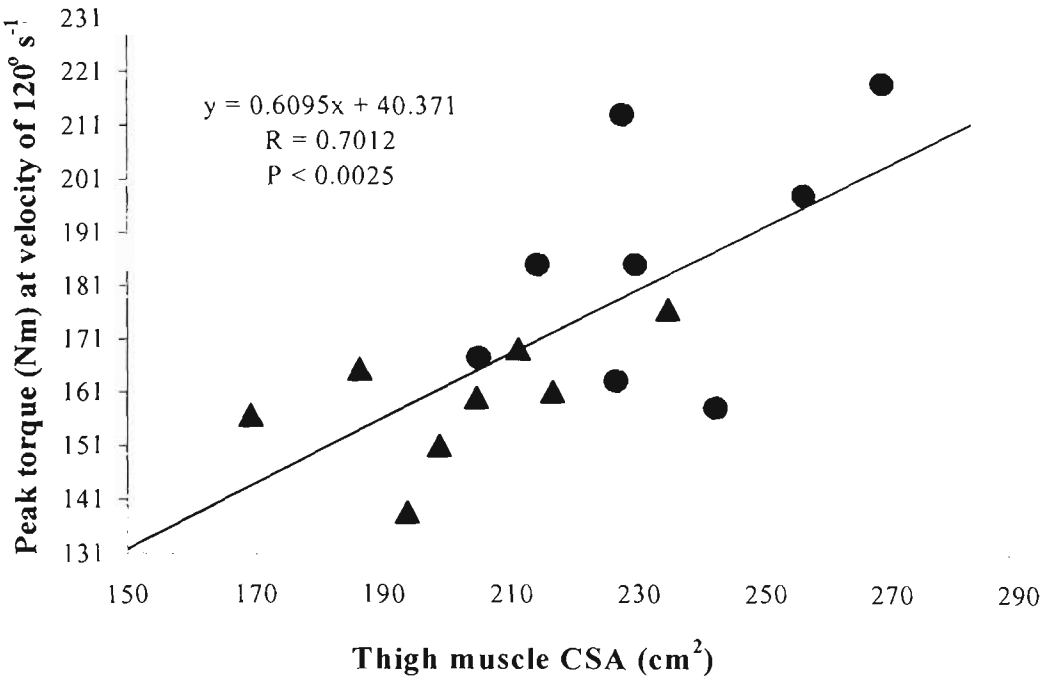


Figure 6.4. Regression of Thigh CSA and Total Work during Biodex Dynamometer Test

● Resistance-trained subjects (*n* = *8*); ▲ Untrained controls (*n* = *8*); mean value; CSA, cross-sectional area.

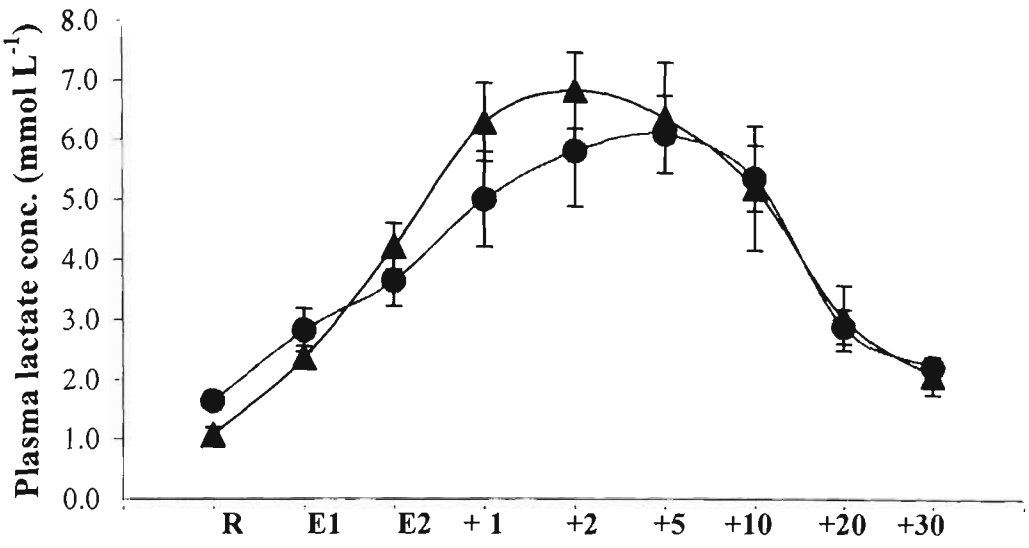


Figure 6.5. Plasma Lactate Concentration during Intense Fatiguing Exercise (Cybex).

● Resistance-trained subjects (n = 8); ▲ Untrained controls subjects (n = 8); mean ± SEM; R, resting; E1, 25 leg repetitions; E2, 50 leg repetitions; +1 to +30, time (min) of recovery.

6.3.3. Muscle Fibre Type

Resistance-trained subjects had similar proportions of type I and type II muscle fibres to the untrained controls (RT, 50.1 ± 4.5% versus UT, 52.7 ± 3.4%). The proportions of type IIa (RT, 40.9 ± 3.4% versus UT, 36.8 ± 1.73%) and type IIb (RT, 9.0 ± 2.7% versus UT, 10.5 ± 3.4%) muscle fibres were also similar between the two groups.

6.3.4. Metabolic Enzyme Activities

When enzyme activity is expressed per unit muscle weight, the activity of mitochondrial enzymes including GDH, CS, KGDH, and HAD, were similar in the RT and the UT groups (Table 6.5). PK and HK activities, however, were significantly higher in the RT group than in

the UT group ($P < 0.05$). PFK (16%; $P = 0.13$) and PHOSPH (20%; $P = 0.14$) activities tended to be higher in the RT group than in the UT group, but were not significantly different between the two groups (Table 6.6). When the activity of the enzymes was expressed in terms of muscle protein content, enzyme activities were not significantly different between the two groups.

Table 6.5. Activity of Muscle Enzymes Expressed Per Unit Muscle Weight

Enzyme	UT	RT
PFK	31.2 ± 0.9	36.3 ± 2.8
PHOSPH	15.6 ± 1.0	18.7 ± 1.7
HK	1.9 ± 0.1	2.4 ± 0.2*
PK	214 ± 16.5	264 ± 16.3*
GDH	0.94 ± 0.06	1.04 ± 0.06
CS	19.4 ± 0.7	18.4 ± 1.0
KGDH	0.78 ± 0.1	0.81 ± 0.1
HAD	4.43 ± 0.3	4.12 ± 0.3

Values are mean ± SEM, expressed as mmol min^{-1} kg^{-1} muscle weight. RT, resistance-trained subjects (*n = 7; CS & GDH n = 8*); UT, untrained subjects (*n = 8; KGDH & HAD n = 7*).
* $P < 0.05$.

Table 6.6. Activity of Muscle Enzymes Expressed Per Unit Muscle Protein

Enzyme	UT	RT
PFK	184.2 ± 7.5	201.6 ± 13.3
PHOSPH	90.7 ± 3.7	104.2 ± 9.6
HK	11.4 ± 1.0	13.1 ± 0.7
PK	1281 ± 136	1478 ± 106
GDH	5.7 ± 0.6	6.2 ± 0.6
CS	115.3 ± 7.3	108.5 ± 9.8
KGDH	4.7 ± 0.7	4.5 ± 0.8
HAD	26.5 ± 1.7	22.9 ± 1.6

Values are mean ± SEM, expressed as mmol min^{-1} g^{-1} muscle protein. RT, resistance-trained subjects (*n = 7; CS & GDH n = 8*); UT, untrained subjects (*n = 8; KGDH & HAD n = 7*).

6.3.5. Muscle Metabolites

The results of metabolite analyses are listed in Table 6.7. With the exception lactate and glycogen, all muscle metabolite concentrations were adjusted to the peak total creatine concentration for each subject. The average correction to muscle metabolites made in this way were 9% in RT subjects and 5% in UT controls. The concentration of ATP measured using HPLC in fatigued muscle compared with rest in samples was reduced in the UT group. The ATP concentration of fatigued samples was not statistically different compared with resting muscle in the RT group ($P > 0.05$). However, the ATP concentration determined by the enzymatic method in fatigued muscle was decreased in both UT and RT groups compared with rest muscle samples ($P < 0.01$). There were no differences between the two groups in both the resting muscle and the fatigue muscle samples.

A lower PCr concentration ($P < 0.01$) and a higher Cr concentration ($P < 0.01$) were present

in fatigued muscle compared with the resting muscles in both groups. The PCr concentration of the resting muscle in RT group was higher than UT group ($P < 0.01$). The total creatine (TCR) was also higher in the RT than UT group ($P < 0.05$). The concentration of muscle IMP at fatigue was increased in the UT ($P < 0.01$) and in the RT group ($P < 0.05$). Fatiguing exercise also induced that muscle lactate concentration was increased in the two groups ($P < 0.01$). Muscle glycogen concentration in fatigued muscles was reduced in UT group ($P < 0.01$) and in the RT group ($P < 0.05$). However, the concentration of Cr, IMP, lactate and glycogen were not different either at rest or at fatigue when comparing the two groups. The remaining metabolites ADP and AMP were not significantly different in the fatigued muscle samples compared with the resting muscles in the two groups. There were also no differences in ADP and AMP between the two groups. The ratio of ATP/ADP, TAN and TAN+IMP in the RT and UT groups were also similar in the resting and fatigue conditions.

Table 6.7. Resting and Fatigued Skeletal Muscle Metabolites

Metabolites	UT		RT	
	Resting	**Fatigue**	**Resting**	**Fatigue**
ATP (HPLC)	24.7 ± 0.9	21.4 ± 1.4**	25.9 ± 1.6	22.5 ± 1.4
ATP (Enz.)	25.7 ± 0.9	19.5 ± 1.7**	26.6 ± 0.8	20.3 ± 2.0**
ADP	2.8 ± 0.2	2.9 ± 0.5	3.2 ± 0.3	3.3 ± 0.3
AMP	0.13 ± 0.02	0.14 ± 0.02	0.11 ± 0.03	0.15 ± 0.03
IMP	0.08 ± 0.01	3.26 ± 0.84**	0.10 ± 0.02	2.09 ± 0.74*
PCr	93.3 ± 2.3	35.8 ± 3.5**	105 ± 2.7‡	42.6 ± 3.1**
Cr	42.0 ± 2.6	99.5 ± 4.6**	44.8 ± 3.3	107 ± 3.0**
TCR	135 ± 3.9	135 ± 3.9	150 ± 3.7†	150 ± 3.7†
Glycogen	468 ± 34	356 ± 31**	478 ± 27	358 ± 24*
Lactate	8.04 ± 0.8	91.6 ± 10.0**	10.1 ± 0.8	85.1 ± 8.9**
ATP/ADP	9.3 ± 0.8	8.5 ± 1.2	8.4 ± 0.7	7.2 ± 0.7
TAN	27.6 ± 0.8	24.5 ± 1.8	29.2 ± 1.7	26.0 ± 1.5
TAN+IMP	27.7 ± 0.8	27.7 ± 1.8	29.3 ± 1.7	28.0 ± 1.3

Values are mean ± SEM, expressed in terms of mmol kg^{-1} dry muscle, except ATP/ADP. RT, resistance-trained subjects ($n = 8$); UT, untrained subjects ($n = 8$); ATP (HPLC), measured by HPLC; ATP (E), ATP measured enzymaticly; TCR, total creatine (PCr + Cr); TAN, total adenine nucleotides (ATP + ADP + AMP) were determined by HPLC; The ATP/ADP ration was calculated from HPLC determined ATP and ADP. TCR, total Creatine (PCr + Cr); * $P < 0.05$, ** $P < 0.01$ comparing the rest and the fatigue muscle samples; †, $P < 0.05$, ‡ $P < 0.01$ comparing the two groups.

6.3.6. MAPR

Comparing the two groups, there was no difference in the muscle sample weight, mitochondrial suspension protein concentration, the yield of mitochondrial suspension protein in muscle and the amounts of mitochondrial suspension protein used for the MAPR assays. The proportions of intact mitochondria were also similar between the two groups. These results are listed in Table 6.8. There were no differences in the MAPR in the presence of various substrate combinations between the UT and the RT groups, irrespective of whether the MAPR was expressed per unit mitochondrial suspension protein (Table 6.9) or per unit muscle weight (Table 6.10).

Table 6.8. Mitochondrial Protein and Mitochondrial Yield

Mitochondrial Preparation	UT	RT
Muscle sample wet weight (mg)	31.3 ± 2.2	29.6 ± 3.1
Protein concentration of mit. Suspension (mg ml^{-1})	0.59 ± 0.08	0.66 ± 0.08
Mit. Suspension protein in assay (ng ml^{-1})	23.5 ± 3.2	26.9 ± 3.1
Yield of mit suspension protein in muscle (g. protein kg^{-1} muscle)	3.64 ± 0.3	4.72 ± 0.7
Yield of Mit from GDHim/GDHt (%)	19.49± 1.8	26.9 ± 4.0

Values are mean ± SEM. RT, resistance-trained subjects (n = 7); UT, untrained subjects (n = 8); Mit, mitochondria; GDHim. intact mitochondrial GDH activity; GDHt, total GDH activity.

Table 6.9. MAPR Expressed Per Unit Mitochondrial Suspension Protein

Substrates	UT	RT
P + M	0.37 ± 0.04	0.37 ± 0.04
PC + M	0.27 ± 0.03	0.26 ± 0.03
S + R	0.38 ± 0.05	0.37 ± 0.02
A-KG	0.40 ± 0.05	0.39± 0.05
PPKM	0.59 ± 0.06	0.59 ± 0.07

Values are mean ± SEM, expressed as mmol min^{-1} g^{-1} protein of mitochondrial suspension. RT, resistance-trained subjects (*n* = *7*); UT, untrained controls (*n* = *8*).

Table 6.10. MAPR Expressed Per unit Muscle Weight

Substrates	UT	RT
P + M	6.86 ± 0.53	6.56 ± 0.68
PC + M	4.95 ± 0.46	4.62 ± 0.43
S + R	7.01 ± 0.72	6.61 ± 0.34
A-KG	7.36 ± 0.71	6.76 ± 0.51
PPKM	10.86 ± 0.82	10.17 ± 0.65

Values are mean ± SEM, expressed as mmol min^{-1} kg^{-1} muscle weight. RT, resistance-trained subjects (*n* = *7*); UT, untrained controls (*n* = *8*).

6.4. DISCUSSION

6.4.1. Major Findings

The present study demonstrated the long-term resistance-trained subjects have a larger thigh muscle mass compared with the UT controls. The thigh muscle peak torques at angular velocities of 60 through to 360 ^{o} s^{-1} and total work measured by the Biodex dynamometer

were significantly higher in the RT than the UT group. The resistance-trained subjects, however, had the same exercise endurance (fatigue index) as the controls. Resistance-trained subjects exhibited a higher concentration of PCr and higher activities of the enzymes HK and PK in resting muscle samples compared with the controls. The proportion of muscle fibres, oxidative enzyme activities, and MAPR were similar in the RT group and the UT group. Muscle metabolites in fatigued muscle samples exhibited lower ATP, PCr, and glycogen and higher IMP, Cr and Lactate concentrations, but were not different between the two groups.

6.4.2. Muscle Function

In the present study, the peak isokinetic torques at angular velocities of 60 through to 360 $^{\circ}$ s^{-1}, were significantly higher in the RT subjects than in the UT group. The total work in 50 repeated maximal knee extensions, measured on the Biodex dynamometer, was also higher in the RT than the UT group. These parameters indicated that the skeletal muscle contractile function was superior in the RT group compared with the UT because the torque-velocity relation is the best method to characterise the contractile behaviour of intact muscle (Gülch 1994). Increased muscle strength and force output are the most common features of long-term resistance-trained subjects (Thorstensson et al 1976b; Houston et al 1983; Alway et al 1988; McCall et al 1996; Moss et al 1997; Goreham et al 1999). Improvement of skeletal muscle function by resistance training may be related to neuromuscular adaptation (Komi 1978; Houston et al 1983; Hakkinen et al 1985; Housh and Housh 1993; Moss et al 1997) and enlarged muscle mass or muscle fibre area (MacDougall et al 1979; Tesch et al 1990; Kraemer et al 1995; Narici et al 1996; Moss 1997). Repented metabolic adaptations include enhanced activity of metabolic enzymes (Komi et al 1978; Costill et al 1979; Côté et al 1988) and increased ATP, PCr and glycogen concentrations in resting muscle (MacDouall et al 1977). Resistance training also reduced the substrates (PCr and glycogen) utilisation and

decreased the accumulation of IMP and lactate during submaximal exercise (Goreham et al 1999). Examples of training details, muscle function and muscle histological alterations in healthy subjects after resistance training and strength training were listed in Table 6.11. In the present study, the thigh CSA was positively correlated with the muscle torque at 60 through to 360^{o} s^{-1} velocities and positively correlated with total work output during intense knee exercise (Biodex). These observations are consistent with suggestions that resistance training-induced thigh muscle enlargement may be one of key factors improving muscle function in the leg. In the present study, muscle PCr concentration and enzyme HK and PK activities in resting muscle were also higher in the RT group compared with the UT group. These alterations may also contribute to the improvement of muscle function and their physiological significance will be discussed subsequently.

Table 6.11. Examples of the Effects of Resistance Training and Strength Training on Skeletal Muscle Function, Metabolism and Histology

Sources	Muscle	Training	Muscle Strength (%)	Muscle CSA (%)				Metabolic Enzyme & Metabolism (%)	NMA (%)
				I	IIa	IIb	Other		
MacDouall et al 1977	TS	5m.	↑35**				↑11*	↑ATP 10*, PCr 3, ADP 29*, & glycogen 21*.	
Costill et al 1979	VL	7w.	↑ 14*	↔	↔	↔		↑ MDH 14*, SDH 11*, PHOSPH 8*,PFK22* CPK15* & MK 13*	
Komi et al 1978 (Monozygous twin)	VL	12w.	↑ 20**.				↔	↑ MDH 30*, SDH 32*, HK 8*, LDH 8* & CPK 16*	IEGM ↑ 38% l trained leg MIF ↑ 11% in untrained leg
Gettman et al 1979	VL	16w.	↑ 18** ↑ 13**.				↔	$\dot{V}O_2$ max ↑ 10*	
Houston et al 1983	VL	12w.	↑ 39 to 60*		↑21	↑ 18		SDH,HAD, HK, CK, LDH ↔	↑ 12 to 37 in untrained leg
Hakkinen et al 1985	VL	24w.	↑ 27*	↑ 21	↑21				IEMG ↑*
Lüthi et al 1986	VL	6w.	↑ 18%*				↑8*	Mitochondrial volume ↔	
Côté et al 1988	VL	10w	↑ 54.	↔	↔	↔		↑ MAD 18*, HAD 25*, KGDH 89*. HK 17*.	
Kraemer et al 1995	VL	12w.	↑ 30* to ↑ 34*		↑74*	↑90*	↑21*		
Narici et al 1996	VL	6m.	↑30* & ↑21**				↑19*		IEMG ↔
Moss et al 1997	VL	9w.	↑ 6.6* to 15 **.				↑28*		Untrained arm ↑ 7*
Goreham et al 1999 #	VL	12w.	↑ 7.8* to 57*.				↑*	↑ IMP* ↑Cr* ↓PCr*	

IEMG, integrated electromyography activity; MIF, maximal isometric force; NMA, neuromuscular adaptation; TS, triceps surae; VL. vastus laterals; ↔, unchanged. #, during submaximal exercise; * $P < 0.05$; * $P < 0.01$.

6.4.3. Muscle Endurance Performance

Resistance training increases muscle strength and force output, but it may not increase the resistance to muscle fatigue during exercise. In the present study, the RT subjects did not achieve a longer duration of exercise or higher $\dot{V}O_2$ peak in the incremental bicycle test, nor did they achieve a lower fatigue index during the 50 knee extension intense exercise tests. These results indicate that exercise fatigability was not different between the two groups.

A number of previous studies have reported that $\dot{V}O_2$ peak was not affected (Hickson et al 1988; Marcinik et al 1991; Rube and Secher 1991; Kraemer et al 1995; Goreham et al 1999) or only increased about 4% (Hickson et al 1980) to 10% (Gettman et al 1979), by a period (12 to 16 weeks) of resistance training and strength training. Bishop and Jenkins (1996) demonstrated that 6 weeks resistance training did not improve indices of endurance ability as measured by exercise time to exhaustion and $\dot{V}O_2$ peak. In contrast, Hickson et al (1980) examined the effect of 10 weeks of strength training on endurance performance during exercise bouts of 4 to 8 min duration against progressively increasing workloads. They found that exercise endurance time to exhaustion was significantly increased by about 47% while cycling and 12% while running. In the subsequent study, Hickson et al (1988) also demonstrated that 10 weeks resistance training significantly increased endurance performance as measured by exercise time to exhaustion during cycling at 80% $\dot{V}O_2$ max. Similarly, Marcinik et al (1991) observed the effects of 12 weeks strength training on cycling time to exhaustion at 75% of $\dot{V}O_2$ max exercise intensity. They found that the strength training result in no change in $\dot{V}O_2$ peak, but enhanced cycling endurance by ~33% in a group of subjects. They also demonstrated that $\dot{V}O_2$ at LT was 12% higher in these trained subjects during exercise.

Resistance training induced exercise endurance increase may be related to increased muscle

strength and muscle mass (Hickson et al 1980; 1988), increased muscle fibre capillarisation (Green et al 1998) or increased muscle glycogen concentration (MacDougall et al 1977). It is also possible that resistance training improves muscle mitochondrial function, as indicated by increased mitochondrial enzyme activities (Komi et al 1978; Costill et al 1979). These changes may result in an increase in lactate threshold (Marcinik et al 1991) and a reduction of lactate production during submaximal exercise (Goreham et al 1999). In the present study, the resistance-trained subjects did not show lesser fatigue index levels in intense exercise compared with the untrained controls. These results, however, have do not mean that long-term resistance training may not be implicated in endurance capacity in intense exercise. The individual variances may affect the observations in the present study due to the cross-sectional study mode that was used. Consequently, the effects of long-term resistance training on exercise endurance in high intense exercise need to be investigated using a longitudinal study mode in future.

6.4.4. Muscle Cross-Sectional Area and Fibre Type Proportion

Enlarged muscle bulk, indicated by the CSA of muscle, is one of the common characteristics in resistance-trained subjects (Tesch et al 1987; Tesch 1988; Narici et al 1996; see Table 2.3). Resistance training enhances muscle mass which may be related to the fact that training stimulates skeletal muscle protein synthesis (MacDougall et al 1995; Ferrando et al 1997). The hypertrophied muscles display greater cross-sectional area in the two main types of fibres (MacDougall et al 1980; Staron et al 1990; Hather et al 1991; Wang et al 1993; McComas 1994; McCall et al 1996; Green et al 1998). This muscle fibre hypertrophy improved the muscle function (see Table 6.11). Although the muscle fibre area was not measured in the present study, there is indirect evidence that muscle mass was greater in the RT group than UT. As indicated in Table 6.1, the thigh CSA (excluding the thigh skinfold) was 16% greater

in RT compared with UT, since thigh CSA represents, at least, 96% of the muscle cross-section area and bone area only ~ 4% if excluding the subcutaneous fat (Kanehisa et al 1994).

In the present study, the proportions of type I, II and IIb fibres in the resistance-trained subjects were the same as the UT controls. The same results were also reported by a number of previous studies for both short (7 to 12 weeks) resistance training (Costill et al 1979; Houston et al 1983; McCall et al 1996) and long-term (~10 years) strength-trained subjects (Alway et al 1988). In contrast, it has been reported that strength trained (average 8.6 years) subjects (Schantz and Källman 1989) have a higher ($P < 0.05$) proportion of type I fibres (~67% *vs.* 59% in controls) in the deltoid muscle compared with the controls. Staron et al (1984) demonstrated that weight lifters (> 3 year trained) also had significantly higher proportion of type I fibres in vastus lateralis muscle (~38% *vs.* 23% in controls). It should be point out, however, that the proportion of type I fibres (23%) in the vastus lateralis muscle in normal subjects is around 50% (Costill et al 1975; see Section 2.3.3). Hence, the proportion of type I fibres reported in the study by Staron et al (1984) is very much lower than the typical value for this type fibre in that muscle. In most reports, resistance training and strength training did not affect the proportion of type I fibres, but the proportion of type IIa fibres was increased (MacDougall et al 1980; Staron et al 1991; Fry et al 1994; Kraemer et al 1995; Carroll 1998; Green et al 1998). Numenous studies have shown that 6 to 12 week resistance and strength training induces muscle fibre transformation from type IIb (decrease from ~15%-25% to 8% - 0%) to IIa fibres (decrease from 23% - 45% to 33% - 60%) in studies utilising muscle histochemistry (Hather et al 1991; Staron et al 1990; 1991; 1994; Kraemer et al 1995; Green et al 1998). Adams et al (1993) demonstrated that heavy resistance training for 19 weeks significantly reduced myosin heavy chain isoform IIb contents by ~12% and increased IIa by 12% in vastus lateralis muscle. Fry et al (1994) also demonstrated that muscle MHCIIa fibre increased by 12% and MHCIIb fibre proportion decreased by 61% after 9 weeks high intensity resistance training. Resistance training-induced reduction in the proportion of type

IIb muscle fibre became statistically different as early as 2 weeks in women and 4 weeks in men after commencement of training (Staron et al 1994).

The cross-sectional design used in the present study, is not the best method to investigate the alteration in muscle fibre types. The reason is that the proportion alterations in type IIa and IIb fibres are usually about 12 to 15% after a period training, but the individual variance of the proportion of fibre types may beyond these ranges in human muscle. For example, the reported type IIa fibre proportions in the vastus lateralis muscle ranged from 28% to 44% in untrained men from 19 to 33 years of age (Hather et al 1991; Green et al 1998). The type IIb fibre proportions were from 6.3% (Rube and Secher 1990) to 43% (Staron et al 1984) in 30-year-old untrained men. Therefore, a longitudinal study is preferable for observation of muscle fibre changes following resistance training.

6.4.5. Muscle Glycogenolytic and Glycolytic Enzyme Activities

In the present study, the activities of enzymes HK and PK per unit muscle weight were significantly higher in the RT group than the UT group. The high (17%) HK activity in resistance trained subjects in the present study was consistent with the increase of 10% to ~25% of HK activity in other studies (Komi et al 1978; Côté et al 1988; Wang et al 1993). The activity of HK in muscle has also been reported to be unchanged (Houston et al 1983; Tesch et al 1990; Green 1999) and decreased (Tesch et al 1987) after resistance or strength training. HK is the first "non-equilibrium" enzyme in glycolysis (excluding glycogenolysis - PHOSPH) and it catalyses the conversion of glucose to glucose 6 phosphate (see Figure 2.2). The role of HK in heavy resistance exercise is considered to be of minor importance by Tesch et al (1987), since glycogenolysis is the main source of glycolytic flux in this form exercise. Consequently, the effect of HK on exercise performance is not clear.

The present study also found that the activity of PK was ~23% higher in RT than UT. PK is

also a "non-equilibrium" enzyme in glycolysis and it catalyses the conversion of phosphoenolpyruvate to pyruvate. High PK activity should increase the glycolytic rate thus producing more pyruvate which will provide for an expansion of the pool of TCA cycle intermediates in muscle or alternately, generate increased lactate production.

Increased PHOSPH activity in muscle by 8% to 16% after 7 to 12 weeks resistance training were also reported by Costill et al (1979) and Green et al (1999), respectively. As a "non-equilibrium" enzyme, PHOSPH catalyses the conversion of glycogen to glucose-1-phosphate, therefore, high activity of PHOSPH would enhance the glycogenolytic pathway in muscle of resistance-trained subjects. This adaptation would enhance metabolism and muscle performance during maximal exercise. In the present study, however, the activity of PHOSPH was not higher in the resistance-trained subjects compared with the controls.

It is known that PFK is a key rate limiting "non-equilibrium" enzyme for the regulation of glycolysis (Campbell et al 1987). In the present study, the PFK activity expressed per unit muscle weight was 16% higher (P = 0.13) in the RT group compared with the UT group. Although not significant, these results suggest a tendency for higher PFK activity in the RT than the UT. The low subject numbers plus normal assay variability suggest the possibility of a type II error. This higher PFK activity would be consistent with the increased glycolytic capacity reported in this study. Nevertheless, unchanged activity of PFK in skeletal muscle has been reported in short-term (12 to 18 weeks) resistance training (Houston et al 1983; Wang et al 1993; Green et al 1999) and in long-term resistance-trained subjects (Schantz and Kallman 1989).

The result of enzyme activities in the present study suggests that the rate of glycogenolytic and glycolytic reactions may be partially accelerated in the long-term resistance-trained subjects.

6.4.6. Resting Muscle Metabolism

In the present study, resistance trained subjects have shown a higher PCr and therefore higher TCr content in the resting muscle compared with the controls, which suggests that resistance training increased the level of phosphagen storage in resting muscle. The levels of ATP, Cr and glycogen in resting muscle were not significantly different between the two groups in the present study. The results are somewhat inconsistent with the study of MacDougall et al (1977). They reported that 5 months of heavy resistance training led to significant increases in concentrations of ATP, PCr and glycogen in the resting triceps brachii muscle (see Table 6.11). The different results may be due to the fact that the triceps brachii muscle and vastus lateralis muscle are very different muscle groups and have different muscle characteristics. The leg muscles, like vastus lateralis, are weight-bearing muscles which are frequently active, but arm muscle are used less for endurance activity in most people. Therefore, the arm muscles may respond more readily to exercise training than leg muscle (Turner et al 1997). On the other hand, the triceps brachii muscle has higher proportion (~68% to ~71%) of muscle type II fibre (MacDougall et al 1980; 1982) compared with type II fibre (~37% to ~55%) of vastus lateralis muscle (Costill et al 1979; Essén-Gustavsson and Borges 1986). It has been demonstrated that resistance training is more effective in increasing type II fibre area (Staron et al 1984; Schantz and Kallman 1989). Consequently, this form of training is likely to produce more substantial changes in muscle metabolites in arm muscle.

The mechanism that enabled the metabolic adaptation in resistance-trained subjects is still not completely explained. MacDougall et al (1977) suggested that muscle repeated bouts of PCr depletion-repletion might lead to muscle increasing uptake of creatine. The higher PCr concentration may be one of the factors enhancing exercise performance of leg muscles in the RT subjects in the present study. Vandenberghe et al (1997) demonstrated that long-term (5 & 10 weeks) creatine supplementation for resistance training subjects significantly increased PCr concentration by 6% and the isokinetic dynamic torque by > 60% in arm muscle during

maximal intermittent exercise. Their study also showed that creatine supplementation for ten week significantly increased leg maximal muscle strength. Maganaris and Maughan (1998) also reported that short-term (5 days) creatine supplementation increased MVC by ~10% and isometric endurance of intensities at 20% through to 80% of MVC. It is apparent, therefore, that increased intramuscular PCr content may improve muscle exercise performance.

6.4.7. Muscle Metabolism in Fatigue

A comparison of the muscle metabolites between the resting and fatigued muscles in the present study has shown that intense leg exercise significantly reduced ATP, PCr and glycogen concentrations and that IMP, lactate and Cr accumulated in both groups. Except for IMP, Tesch et al (1986) also reported similar metabolite changes in muscle after intense resistance exercise. Cellular mechanisms of muscle fatigue have been studied extensively and there are numerous reviews in this area (Westerblad et al 1991; Fitts 1994). Cellular mechanisms which have been implicated in muscle fatigue include alterations to excitation-contraction coupling (Westerblad et al 1991; Sahlin 1992; Fitts 1994), membrane Na^+-K^+ ATPase (Green 1998), alterations of cell ultrastructure (Armstrong et al 1991) and disturbances in metabolism (Sahlin 1992; Fitts 1994; Sahlin et al 1998).

Muscle cell excitation and contraction require ATP to support Na^+-K^+ATPase, Ca^{2+}ATPase and actinomyosin ATPase. Therefore, muscle cells have to maintain adequate ATP levels in order to maintain normal function. It is well known that skeletal muscle ATP and PCr storage are very limited and oxygen delivery to muscle is also initially inadequate to meet the energy demands of muscle contraction in intense exercise. In these situations, three processes of anaerobic metabolism (phosphocreatine kinase, adenylate kinase and anaerobic glycogenolysis and glycolysis) become the main sources of maintenance of ATP levels in muscle (see Section 2.1.2). With intense muscle contraction, ATP utilisation rate is greater

than ATP production rate and these anaerobic reactions are no longer able to supply enough ATP for muscle activities, ATP reduction occurs and this is followed by muscle fatigue. The muscle ATP is usually decreased by ~10 to ~40% at fatigue (Sahlin 1992) and this is associated with decreased glycogen and accumulation of Cr, H^+, IMP and lactate in muscle (Sahlin et al 1990). However, the role of ATP in muscle fatigue is still not clear, because the ATP is not decreased at fatigue to the levels which have been considered to limit muscle contractions (Fitts 1994). Reduced ATP has been linked with reduced Ca^{2+} release from the sarcoplasmic reticulum in mouse muscle (Allen et al 1996). However, Fitts (1994) has pointed out that as an important energy store, PCr acts to buffer cellular ATP and has suggested that PCr is likely to be one of the limitations to muscle performance during intense, fatiguing, short-lasting muscle contractions (Meyer et al 1986).

Accumulation of lactate in muscle is also associated with intracellular acidosis (Sahlin et al 1978) which can inhibit force (Metzger and Fitts 1987) and is a possible cause of muscle fatigue (Hermansen and Osnes 1972; Cooke et al 1988). These studies were performed at room temperature and showed a substantial decrease in muscle force of 30%. More recent study, however, found that acidification did not significantly affect on maximum shortening velocity in single muscle fibre of mouse at 32C (Westerblad et al 1997). Lactate accumulation per se has been reported to decrease muscle force (Spangenburg et al 1998) and Ca^{2+} release from the sarcoplasmic reticulum (Favero et al 1997).

Elevation of IMP in fatigued muscle indicates an imbalance of energy metabolism during exercise. It has been suggested that IMP accumulation might be result in the impairment of contractile process (Sahlin et al 1990). There is, however, no evidence in that IMP directly induces muscle fatigue.

Glycogen depletion occurs in fatigue muscle after repeated high intense exercise (Bangsbo et al 1992a; Blomstrand and Saltin 1999). The role of glycogen in muscle exercise to fatigue is dependent on the exercise intensity and duration. In intense exercise, comparable to that

performed in the present study, glycogen availability does not appear to be a limitation to performance (Bangsbo et al 1992a; Hargreaves et al 1997). More recently, a study by Balsom et al (1999) demonstrated that low muscle glycogen levels (180 mmol L^{-1} kg^{-1} dw) reduced exercise performance during both short-term and more prolonged high intensity intermittent exercise. In the present study, however, the mean muscle glycogen levels were 478 *vs.* 470 mmol L^{-1} kg^{-1} dw before and 358 *vs.* 368 mmol L^{-1} kg^{-1} dw after exercise in the RT and UT, respectively. On this bases it would appear that muscle glycogen levels were not likely to relate to the exercise fatigue.

In addition, aerobic metabolism also is important in intense exercise (MedbØ and Tabata 1989). The role of mitochondrial capacity in fatigue, however, is not clear and previous studies have been reviewed in Section 2.3.4.4.

6.4.8. Mitochondrial Functions in Muscle

In the present study, the activity of four regulatory mitochondrial enzymes was not statistically different between the two groups. These results are consistent with the lack of alteration in activity of enzyme CS and HAD in subjects after short-term (18 weeks) training (Wang et al 1993) and long-term (8 years) training (Schantz and Källman 1989). The activity of KGDH in the RT subjects in the present study was inconsistent with the study of Côté et al (1988). They showed that repeated concentric isokinetic strength training significantly increased the activity of KGDH in muscle. The effect of resistance training on activity of GDH in muscle has not been reported previously. In addition to measuring activity of oxidative enzymes, the present study, however, through the measurement of MAPR is able to assess the total functional capacity of mitochondria in skeletal muscle.

Similar to the oxidative enzyme activities, in the present study, the values of MAPR with various substrates in terms of mitochondrial suspension protein were the same between the two groups. The values of MAPR with various substrates per unit muscle weight were not

different in the RT group compared with the UT controls. These results, however, can not exclude the possibility that resistance training improves mitochondrial capacity in skeletal muscle. Once again, the present study was a cross-sectional study, therefore, the individual variations may offset the alterations. A longitudinal study may reduce this kind of error to demonstrate the alteration in muscle mitochondrial capacity after resistance training. Longitudinal studies are readily conducted for investigating the effects of a short-term training programs, but are more difficult for long-term (years) training programs. Therefore, cross-sectional studies, such as the present study and these conducted by Staron et al (1984), Lüthi et al (1986), and Alway et al (1988), may still a useful method for investigating resistance training.

Previous studies have given an unclear picture of the effects of resistance training on mitochondria in muscle (see Section 2.3.4.3). It is possible, however, that change in muscle mass which occurs as a result of resistance training may mask any changes in mitochondrial enzyme activity and mitochondrial volume density (see Table 2.3). In most studies, increases in muscle fibre area and muscle CSA were associated with a decreased or unchanged mitochondrial enzyme activities and/or mitochondrial volume density (MacDougall et al 1979; Lüthi et al 1986; Tesch et al 1987; Alway et al 1988; Schantz & Kallman 1989; Wang et al 1993; Green et al 1999). In contrast, the mitochondrial volume density and enzyme activities were increased when the muscle fibre cross-sectional area or CSA was unchanged (Kimo et al 1978; Costill et al 1979; Côté et al 1988). Wang et al (1993) have demonstrated that the absolute volume of mitochondria significantly increased in type I and IIa muscle fibres by 18% and 25%, respectively, after 18 weeks high-repetition resistance training. The percentage volume of mitochondria after training, however, remained the same as before the training due to a 20% increase in the volume of myofibrils in type I fibres and a 26% increase in type II fibres. The volume of mitochondria in type IIb fibres increased by 6% and was associated with 6% increase in the volume of myofibrils in those subjects. In the present

study, the thigh muscle mass was greater in the RT subjects. MAPR and mitochondrial enzyme activities were the same in RT and UT groups. Hence, the total mitochondrial capacity of the value labialisation (mass × activity) would be greater in resistance-trained subjects.

It has been demonstrated that endurance training increases mitochondrial capacity in skeletal muscle (see Section 2.3.4.2). In the present study as result of long-term resistance training, mitochondrial enzyme activities and function appear to have increased to the same extent as muscle mass. It should be noted that the control subjects in the present study were not physical inactive. Previous studies by Ingemann-Hansen and Halkjaer-Kristensen. (1983; 1985b) have demonstrated resistance training significantly increased in the activity of mitochondrial enzymes in patients whose muscle had been wasted as result of long-term bed rest. Consequently, resistance training is likely to be more effective in improving the muscle oxidative capacity for hypotrophic muscle. Mechanisms by which resistance training influence mitochondrial capacity are unknown and need to be investigated.

6.5. CONCLUSION

In conclusion, compared with untrained subjects, this study demonstrated that resistance-trained subjects have a significantly larger thigh cross-sectional area (CSA) which indicates that their thigh muscles are larger than controls. The resistance-trained subjects also exhibited higher glycolytic enzyme HK and PK activities per unit muscle weight and higher PCr (and total creatine — TCR) concentration in leg muscle compared with the controls. These factors may contribute to the high leg muscle torque output in the RT subjects. During intense resistance exercise muscle ATP, PCr and glycogen decreased and muscle Cr, lactate and IMP increased in both groups and there were no differences between the two groups. Mitochondrial oxidative enzyme activities and MAPR in skeletal muscle were also not

different between the two groups. Since the muscle mass was significantly enlarged in resistance-trained subjects, the total muscle mitochondrial capacity in resistance-trained subjects would be greater.

CHAPTER 7. SUMMARY, CONCLUSIONS AND RECOMMENDATION FOR FUTURE RESEARCH

7.1. SUMMARY

This thesis comprises three studies. The first study examined the skeletal muscle fibre type distribution, resting muscle metabolites, activities of muscle mitochondrial enzymes, glycolytic enzymes and glycogenolytic enzyme and mitochondrial ATP production rate (MAPR) *in vitro* in a group of lung transplant (LTx) recipients and a group age and sex matched control subjects. Exercise performance was tested in these subjects as well. The second study determined the effect of the immunosuppressive drug, cyclosporine A (CyA), on MAPR *in vitro*. The final study examined the thigh muscle fibre type proportions, muscle metabolic enzyme activities, resting and fatigue muscle metabolites and MAPR in 8 long-term resistance trained (RT) subjects and 8 untrained (UR) subjects. The exercise performance and thigh muscle functions were also examined in RT and UT subjects. The major findings in these studies as summarised below.

7.1.1. Major Findings in Lung Transplant Study

Chapter 4 is the first study aimed to examine comprehensively factors in skeletal muscle in lung transplant patients impacting up on endurance exercise performance. The major findings in this study were:

- Lung transplant recipients showed a significantly lower exercise performance indicated by shorter exercise duration with a lower peak workrate compared with the controls. During exercise test, $\dot{V}O_2$ peak in the LTx patients was lower than controls and predicted value

(51% of control value and ~ 56% of predicted value). Lactate threshold in these patients was also significantly lower than the controls.

- Histology of skeletal muscle in the LTx patients was characterised by a significantly lower proportion of type I muscle fibres and a significantly higher proportion of type II muscle fibres compared with the controls.
- The resting muscle of LTx patients exhibited a significant lower level of ATP, total adenine nucleotides (TAN) and the ratio of ATP/ADP and a significantly higher level of IMP and lactate compared with the UT controls.
- Mitochondrial enzyme activities, GDH, CS, KGDH and HAD in skeletal muscle of the LTx were significantly lower compared with the controls. In contrast, the muscle glycolytic enzyme PFK activity expressed per unit muscle weight was significantly higher in the LTx patients than the controls. The activity of glycolytic enzyme PK and HK and glycogenlytic enzyme PHOSPH was not different between the two groups.
- Mitochondrial ATP production rate with various substrates in LTx patients was significantly lower than the controls. The low MAPR was apparent either expressed per unit mitochondrial suspension protein content or per unit muscle weight. These indicate there were low Complex I, II and III activities in the LTx patients in the present study.

Lower proportion of type I and higher proportion of type II muscle fibres in the LTx potentially can responsible for the lower oxidative capacity in their skeletal muscle (see Section 2.3.1) and decrease endurance exercise performance. Low activity of oxidative enzymes in muscle of the LTx patients reflects is indicate reduced of the muscle aerobic energy metabolism in these patients. The high IMP and low ATP concentrations in resting muscle in the LTx patients demonstrated an imbalance in energy metabolism. The low mitochondrial ATP production rates in the presence of various substrates reflect a low capacity for oxidation of carbohydrates and fatty acids in muscle mitochondria of the LTx patients. Changes in muscle fibre types, low MAPR and low oxidative enzyme activity in the LTx patients may be the causes of metabolic abnormalities in resting muscle. It has been

demonstrated that the muscle oxidative capacity can affect on endurance exercise performance. Consequently, the low mitochondrial function and muscle fibre changes may be the key factors limiting exercise tolerance in the LTx patients.

The reduction of type I fibre proportion, decreased oxidative enzyme activity and abnormalities of metabolism in skeletal muscle of the LTx patients are similar to the skeletal muscle alteration in patients with chronic pulmonary obstructive disease (Jakobsson et al. 1990; Whittom et al. 1996). The low activity of oxidative enzymes (Jakobsson et al. 1995; Maltais et al. 1996) and abnormal metabolites (Payen et al. 1993; Jakobsson and Jorfeldt 1995; Pouw et al. 1998) are also similar to patients with COPD. This observation suggests that LTx patients may have muscle defects before the operation. The causes of muscle defects in lung transplant candidates may be the muscle immobilisation or disuse due to long-term physical inactivity and/or bed rest (Bloomfield 1997; Ferretti et al. 1997). Hypoxaemia in these patients with end-stage lung disease may be another cause for muscle deconditioning (Jakobsson and Jorfeldt 1995; Payen et al. 1993). After transplantation, pre-existing injury of skeletal muscle which may persist despite successful lung transplantation. It has been previously demonstrated that the LTx patients have a low $\dot{V}O_2$ max and low exercise performance pre-operation, which are not completely recovered to normal levels post operation (Williams et al. 1990; 1992a; 1992b). It is possible that the immunosuppressive drugs, such as steroids (Wilcox et al. 1989; Decramer et al. 1996; Lettéron et al. 1997) and cyclosporine A (CyA) (Fernandez-Sola et al. 1990; Hokanson et al. 1995) may damage the skeletal muscle during post operation recovery. Therefore, the causes of skeletal muscle defects in the LTx patients are complex, probably include the factors occurring pre-operation and post-operative medications.

7.1.2. Major Findings in Cyclosporine A Study

Chapter 5 examined the effect of low (1 μg ml^{-1}) and high (50 μg ml^{-1} & 25 μg ml^{-1}) CyA concentrations on MAPR in skeletal muscles from 8 rats. The major findings in this study are:

- CyA significantly inhibited MAPR in rats skeletal muscle *in vitro* at low CyA (1 μg ml^{-1}) and high (50 μg ml^{-1} and 25 μg ml^{-1}) concentrations.
- MAPR was depressed by -18% to -28% in the presence of CyA at 50 μg ml^{-1} which was a similar extent with CyA at 25 μg ml^{-1} concentration (-20% to -25%).
- Although four different combinations of substrates were employed, the extent of reduction of MAPR was very similar in each combination of substrates. This indicates that CyA inhibition of mitochondrial function is not specific to any particular complex of ETC.

The significantly depressed MAPR *in vitro* at low concentration of CyA (1 μg ml^{-1}) indicates that CyA could be the cause of injury to skeletal muscle mitochondrial function in the LTx patients since the LTx patients have taken CyA for long period post operation. The high CyA concentration in this study may be not related to the clinical situation, because the levels of the drugs in the muscle probability never reach to the levels of 50 and 25 μg ml^{-1} in the LTx patients. The low CyA concentration has shown produced of MAPR of ~10 to 12%. The LTx patients, however, had 16 to 46% lower MAPR compared with the normal subjects. This indicates that CyA may not be the only factor reducing the mitochondrial function and causing limitation of exercise capacity in the LTx patients. Even it is not primary cause of reduced exercise performance, CyA may prevent recovery post operation. Although the mechanism of CyA inhibition of mitochondrial function is unclear, this study demonstrated, however, the effects of CyA on mitochondrial function *in vitro* are not restricted to a single enzyme complex.

7.1.3. Major Findings in the Resistance Training Study

The major findings in this study are:

- There was a significantly larger muscle mass [indicated by the larger average value of the cross-sectional area (CSA)] and less fat (indicated by the small anterior thigh skinfold) in the thigh of resistance-trained subjects compared with the UT controls.
- The thigh muscle peak torques were significantly higher in the RT than the UT group at limb velocities of 60, 120, 180, 240, 300 and 360 (^{o} sec^{-1}) measured by the Biodex dynamometer. The total work of 50 knee extensions in the Biodex dynamometer tests was also higher in the RT than UT. The CSA of thigh muscle was correlated with the thigh muscle peak torque at limb shortening velocities of 60, 120, 180, 240, 300 and 360^{o} sec^{-1}.
- The fatigue indexes measured on the Biodex and Cybex dynamometers were no different between the two groups.
- The resistance-trained subjects had similar proportions of muscle type I, type IIa and type IIb fibres to the untrained controls.
- The glycolytic enzymes PK and HK activities per unit muscle weight were significantly higher in the RT group than in the UT group., in terms of muscle protein content, was significantly higher in the RT subjects compared with the UT controls. The glycolytic enzyme PFK and The glycogenlytic enzyme PHOSPH activities were not different between the two groups.
- Resistance-trained subjects had a significantly higher PCr and TCr contents in resting muscles compared with the UT controls. After high intensity exercise, the fatigued muscle exhibited a significant reduction of ATP, PCr and glycogen content and a significant accumulation of IMP, Cr and lactate, but not different in RT group compared with UT controls.

- Muscle oxidative enzymes GDH, CS, KGDH and HAD activities in the resistance trained subjects were not different from the UT controls. Similarly, MAPR with various substrates in the RT subjects were also not different compared with the UT controls.

This study has indicated that long-term resistance-trained subjects had a larger thigh muscle CSA and improved the muscle function. These trained subjects also had a higher activity of HK and PK in skeletal muscle. However, the physical significance of these enzymes on exercise performance is not clear. The resting muscles in the RT subjects contained higher amounts of PCr and TCr than the UT controls indicating the resistance training increased substrate storage in muscle. Since the muscle mass was significantly lager in the RT group than the UT controls, the total mitochondrial capacity would be greater in the resistance-trained subjects compared with untrained subjects.

In the present study, resistance trained subjects did not show in an improvement of either $\dot{V}O_2$ max or exercise endurance performance during maximal exercise. Nor did demonstrated a reduction of fatigue index during intense leg exercise in the present study. For the resistance training studies, the cross-sectional study mode may be unable to revealing these alterations, including the changes in mitochondrial enzyme activities and MAPR.

7.2. RECOMMENDATIONS FOR FURTHER RESEARCH.

The aetiology of muscle deficiencies and the time course of skeletal muscle alteration in lung transplantation patients are still unclear. The issues raised from this thesis have indicated that the major skeletal muscle defects in the LTx patients may occur pre-operatively and persist post-operation. The thesis also showed that the immunosuppressive medication, CyA inhibits mitochondrial function *in vitro* in rat muscle at 1 $\mu g\ ml^{-1}$ concentration. This concentration, based on calculations from other data, as described in Chapter 5, is likely to be the level found in the muscle of LTx patients on long-term CyA therapy. Therefore, CyA may be a factor in

the reduction the exercise capacity for the LTx patients. The study of resistance trained subjects in this thesis demonstrated that resistance training maintain the oxidative capacity in hypertrophied skeletal muscle. Accordingly, the recommendations for further investigations as below:

- A longitudinal model should be employed to investigate the skeletal muscle deficiencies which are found in the present study in the LTx patients pre-operation and post-transplantation. This study may reveal the time course of the alteration of skeletal muscle in the LTx patients. Effects of different mode of training on the relationships between exercise performance, skeletal muscle function and mitochondrial capacity in LTx recipients should be investigated in the future. Future studies should also examine alterations to mitochondrial DNA in these patients, which may provide important information for these patient therapy and their rehabilitation.
- The skeletal muscle CyA and metabolites of CyA levels should be determined in the LTx patients in order to more precisely quantify whether CyA is at a concentration which will inhibit the mitochondrial function in skeletal muscle. The effects of other immunosuppressive medications, such as FK506, on *in vitro* and *in vivo* the mitochondrial ATP production rates in rats and the LTx patients should be also investigated.
- A longitudinal study of skeletal muscle mitochondrial capacity in resistance-trained subjects may more clearly show the effect of resistance training on mitochondrial capacity for skeletal muscle. Therefore, the effect of resistance training on mitochondrial enzyme activities and MAPR in skeletal muscle should be examined before and after a period of resistance training. Previous studies have shown that resistance training is most effective in improving muscle oxidative capacity in patients with severely wasted muscle. It would be most valuable, therefore, to examine this form of training in patients who have had lung transplantation.

- The mitochondrial morphological alteration during the resistance training period should be investigated. The time course for resistance training induced genetic changes, such as, mRNA for oxidative enzyme synthesis should also be investigated.
- Resistance training is an effective exercise-training mode to enhance muscle mass, strength and power output. Combining resistance training and endurance training has been demonstrated to result in greater improvements in exercise endurance time and muscle strength in a group healthy elderly woman (Ferketich et al. 1998). Accordingly, the effects of a combination resistance and endurance training on the muscle mitochondrial capacity, the muscle function and the exercise capacity for lung transplantation patients should be investigated in the future.

REFERENCES

Adams, G. R., B. M. Hather, K. M. Baldwin, and G. A. Dudley. (1993). Skeletal muscle myosin heavy chain composition and resistance training. *J. Appl. Physiol.* 74: 911-915.

Adelman, R., R. L. Saul, and B. N. Ames. (1988). Oxidative damage to DNA: relation to species metabolic rate and life span. *Proc. Nat. Acad. Sci.* 85: 2706-2708.

Alejandro, D. S. J., and J. Petersen. (1994). Myoglobinuric acute renal failure in a cardiac transplant patient taking lovastatin and cyclosporine. *J. Am. soci. nephro.* 5: 153-160.

Allen, D. G., J. Lannergren, and H. Westerblad. (1997). The role of ATP in the regulation of intracellular Ca2+ release in single fibres of mouse skeletal muscle. *J. Physiol.* 498: 587-600.

Al-Lozi, M. T., A. Pestronk, W. C. Yee, N. Flaris, and J. Cooper. (1994). Rapidly evolving myopathy with myosin-deficient muscle fibers. *Ann. Neurol.* 35: 273-279.

Altschuld, R., C. Hohl, L. Carleb, R. starling, and G. Brierley. (1992). Cyclosporine inhibits mitochondrial calcium efflux in isolated adult rat ventricular cardiomyocytes. *Am. J. Physiol.* 262: H1699-H1704.

Alway, S. E., J. D. MaqcDougall, D. G. Sale, J. R. Sutton, and A. J. McComas. (1988). Functional and structural adaptations in skeletal muscle of trained athletes. *J. Appl. Physiol.* 64: 1114-1120.

Ambrosino, N., C. Bruschi, G. Callegari, S. Baiocchi, G. Felicetti, C. Fracchia, and C. Rampulla. (1996). Time course of exercise capacity, skeletal and respiratory muscle performance after heart-lung transplantation. *Eur. Respir. J.* 9: 1508-1514.

Ames, B. N., M. K. Shigenaga, and T. M. Hagen. (1995). Mitochondrial decay in aging. *Biochimica et Biophysica Acta.* 1271: 165-170.

Anderson, P. (1975). Capillary density in skeletal muscle of man. *Acta Physiol. Scand.* 95: 203-205.

Andreone, P. A., M. T. Olivari, B. Elick, C. E. Arentzen, R. K. Sibley, R. M. Bolman, R. L. Simmons, and W. S. Ring. (1986). Reduction of infectious complications following heart transplantation with triple-drug immunotherapy. *J. Heart Transplant* 5: 13-19.

Aragon, J. J. and J. M. Lowenstein. (1980). The purine-nucleotide cycle. Comparison of the levels of citric acid cycle intermediates with the operation of the purine nucleotide cycle in rat skeletal muscle during exercise and recovery from exercise. *Eur. J. Biochem.* 110: 371-377.

Arellano, F., and P. Krupp. (1991). Muscular disorders associated with cyclosporine. *Lancet* 337: 915.

Armstrong, R. B., G. L. Warren, and J. A. Warren. (1991). Mechanism of exercise-induced muscle fibre injury. *Sports Med.* 12: 184-207.

Assis, S. M. A., J. L. Monteiro, and A. C. Seguro. (1997). L-Arginine and allopurinol protect against cyclosporine nephrotoxicity. *Transplantation* 63: 1070-1073.

Åstrand, P. O., and K. Rodahl. (1977). Physical work capacity. In: *Textbook of work physiology*. McGraw-Hill (ed.). 2nd Edition. New York. Pp. 291-329.

Babcock, G. T., and M. Wikström. (1992). Oxygen activation and the conservation of energy in cell respiration. *Nature* 356: 6367, 301-309.

Bakeeva, L. E., Y. S. Chentsov, and V. P. Skulachev. (1978). Mitochondrial framework (reticulum mitochondriale) in rat diaphragm muscle. *Biochim. Biophys. Acta* 501: 349-369.

Baliga, R., N. Ueda, P. D. Walker, and S. V. Shah. (1997). Oxidant mechanisms in toxic acute renal failure. *Am. J. Kidney Dis.* 29: 465-477.

Ball-Burnett, M., H. J. Green, and M. E. Houston. (1991). Energy metabolism in human slow and fast twitch fibres during prolonged cycle exercise. *J. Physiol.* 437:257-267.

Balsom, P. D., G. C. Gaitanos, K. Soderlund, and B. Ekblom. (1999). High-intensity exercise and muscle glycogen availability in human. *Acta Physiol. Scand.* 165: 337-345.

Bando, K., I. L. Paradis, R. J. Keenan, S. A Yousem, K. Komatsu, H. Konishi, R. A. Guilinger, T. N. Masciangelo, S. M, Pham, J. M. Armitage, R. L. Hardesty, and B. P. Griffith. (1995). Comparison of outcomes after single and bilateral lung transplantation for obstructive lung disease. *J. Heart Lung Transplant* 14: 692-700.

Bando, K., R. J. Keenan, I. L. Paradis, H. Konishi, K. Komatsu, R. L. Hardesty, and B. P. Griffith. (1994). Impact of pulmonary hypertension on outcome after single-lung transplantation. *Ann. Thorac. Surg.* 58: 1336-1342.

Bangsbo, J., P. D. Gollnick, T. E. Graham, C. Juel, B. Kiens, M. Mizuno, and B. Saltin. (1990). Anaerobic energy production and O_2 deficit-debt relationship during exhaustive exercise in human. *J. Appl. Physiol.* 422: 539-559.

Bangsbo, J., T. E. Graham, B. Kiens, and B. Saltin. (1992a). Elevated muscle glycogen and anaerobic energy production during exhaustive exercise in man. *J. Physiol. (Lond)* 415: 205-227.

Bangsbo, J., T. E. Graham, L. Johansen, S. Strange, C. Christensen, and B. Saltin. (1992b). Elevated muscle acidity and energy production during exhaustive exercise in man. *Am. J. Physiol.* 263: R891-R899.

Banner, N. R., M. H. Lioyd, R. D. Hamiton, J. A. Innes, A. Guz, and M. H. Yacoub. (1989). Cardiopulmonary response to dynamic exercise after heart and combined heart-lung transplantation. *Br. Heart J.* 61: 215-223.

Barclay, J. K., and M. Hansel. (1991). Free radicals may contribute to oxidative skeletal muscle fatigue. *Can. J. Physiol. Pharmacol.* 69: 279-284.

Barros, E. J. G., M. A. Boim, H. Ajzen, O. L. Ramos, and N. Schor. (1987). Glomerular hemodynamics and hormonal participation cyclosporine nephrotoxicity. Kidney international. 32; 19-25.

Barth, P. G., H. R. Scholte, J. A. Berden, J. M. Van Der Klei-Van Moorse, I. E. M. Luyt-Houwen, E. T. Van I Veer-Korthof, J. J. VanDer Harten, and M. A. Sobotka-Ploihar. (1983). An X-linked mitochondrial disease affecting cardiac muscle, skeletal musle and neutrophil leucocytes. *J. Neurol Sci.* 62: 327-355.

Bartholomeusz, B, K. J. Hardy, A. S. Nelson, and P. A. Phillips. (1996). Bosentan ameliorates cyclosporin A-induced hypertension in rats and primates. *Hypertension* 27: 1341-1345.

Bass, A. K. Vondra, R. Rath, V. Vitek, J. Teisinger, E. Mackova, S. Sprynarová, and M. Malkovska. (1976). Enzyme activity patterns of energy supplying metabolism in the quadriceps femoris muscle (vastus lateralis). *Pflügers Arch. Ges. Physiol.* 361: 169-173.

Baumann, H., M. Jäggi, F. Soland, H. Howald, and M. C. Schaub. (1987). Exercise training induces transition of myosin isoform subunits within histochemically typed human muscle fibres. *Pflügers Arch.* 409: 349-360.

Baumgartner, W. A., S. Augustine, A. M. Borkon, T. J. Gardner, and B. A. Reitz. (1987). Present expectations in cardiac transplantation. *Ann. Thorac. Surg.* 43: 585-590.

Beaver, W. L., K. Wassweman, and B. J. Whipp. (1985). Improved detection of lactate threshold during exercise using a log-log transformation. *J. Appl. Physiol.* 59: 1936-1940.

Ben Bachir-Lamrini, L., B. Sempore, M. H. Mayet, and R. J. Favier. (1990). Evidence of a slow-tofast fibre type transition in skeletal muscle from spontenously hypertensive rats. *Am. J. Physiol.* 258: R352-357.

Bennett, W. M., E. A. Burdmann, T. F. Andoh, D. C. Houghton, J. Lindsley, and L. W. Elzinga. (1994). Nephrotoxicity of immunosuppressive drugs. *Nephrol. Dial. Transplant* 9 (suppl): 141-145.

Bergstrom, J. (1962). Muscle electrolytes in man. *Scand. J. Clin. Lab. Invest.* 68 (Suppl): 11-13.

Bernardi, P. (1992). Modulation of the cyclosporine A-sensitive permeability transition pore by the proton electrochemical gradient. *J. Biol. Chem.* 267: 8834-8839.

Berthon, P., D. Freyssenet, J. C. Chatard, J. Castells, I. Mujika, A. Geyssant, C.Y. Guezennec and C. Denis. (1995). Mitochondrial ATP production rate in 55 to 73-year-old men: effect of endurance training. *Acta Physiol. Scand.* 154: 269-274.

Biring, M. S., M. Fournier, D. J. Ross, and M. I. Lewis. (1998). Cellular adaptations of skeletal muscles to cyclosporine. *J. Appl. Physiol.* 84: 1967-1975.

Bishop, D., and D. G. Jenkins. (1996). The influence of resistance training on the critical power function & time to fatigue at critical power. *Aust. J. Sci. Med. Sport.* 28: 101-105.

Bjørtuft, Ø., S. Simonsen, O. R. Geiran, J. G. Fjeld E. Skovlund, and J. Boe. (1996). Pulmonary Haemodynamics after single-lung transplantation for end-stage pulmonary parenchymal disease. *Eur. Respir. J.* 9: 2007-2011.

Blakemore, S. J., P. K. Rickhuss, P. W. Watt, M. J. Rennie, and H. S. Hundal. (1996). Effects of limb immobilization on cytochrome c oxidase activity and GLUT and GLUT5 protein expression in human skeletal muscle. *Clin. Sci. Colch.* 91: 591-599.

Blomstrand, E., and B. Saltin. (1999). Effect of muscle glycogen on glucose, lactate and amino acid metabolism during exercise and recovery in human subjects. *J. Physiol.* 514: 293-302.

Bloomfield, S. A. (1997). Changes in musculaoskeletal structure and function with prolonged bed rest. *Med. Sci. Sports Exerc.* 29: 197-206.

Boobis, L. H., C. Williams, and S. A. Wootton. (1982). Human muscle metabolism during brief maximal exercise. *J. Physiol.* 338: 21-22.

Booth, F. W. (1978). Regrowth of atrophied skeletal muscle in adult rats after ending immobilisation. *J. Appl. Physiol.* 44: 225-230.

Borutaite, V., V. Mildaziene, G. C. Brown, and M. D. Brand. (1995). Control and kinetic analysis of ischemia-damaged heart mitochondria which parts of the oxidative phosphorylation system are affected by ischemia? *Biochim. Biophys. Acta* 1272: 154-158.

Bowyer S. L., M. P. LaMohte, and J. R. Hollister. (1985). Steroid myopathy incidence and detection in a population with athma. *J. Allergy Clin. Immunol.* 76: 234-242.

Brand, M. D., L. F. Chien, E. K. Aniscow, D. F. Rolfe, and R. K. Porter. (1994). The causes and functions of mitochondrial proton leak. *Biochem. Biophys. Acta* 1187: 132-139.

Broekemeijer, K. M., M. E. Dempsey, and D. R. Pfeiffer. (1989). Cyclosporine A is a potent inhibitor of the inner membrance permeablity transition in liver mitochondria. *J. Biol. Chem.* 264: 7826-7830.

Brooke, M. H., and K. K. Kaiser. (1970). Three "myosin ATPase" systems: the nature of their pH lability and sulfhydral dependence. *J. Histochem. Cytochem.*18: 670-672.

Brooks, G. A., K. J. Hittelman, J. A. Faulkner, and R. E. Beyer. (1971). Temperature, skeletal muscle mitochondrial functions, and oxygen. *Am. J. Physiol.* 127: 1053-1059.

Brozek, J., F. Grande, J. T. Anderson, and A. Keys. (1963). Densinometric analysis of body composition: revision of some quantitative assumptions. *Ann. N. Y. Acad. Sci.* 110: 113-140.

Brudvig, G. W., T. H. Stevens, and S. T. Chan. (1980). Reactions of nitric oxide with cytochrome c oxidase. *Biochemistry* 19: 5275-5285.

Burke, C. M., A. R. Glanville, J. Theodore, and E. D. Robin. (1987). Lung immunogenicity, rejection, and obliterative bronchiolitis. *Chest* 92: 547-549.

Burke, C. M., J. Theodore, J. C. Baldwin, H. D. Tazelaar, A. J. Morris, C. McGregor, E. D. Robin, and S. W. Jamieson. (1986). Twenty-eight cases of human heart-lung transplantation. *Lancet* 1: 8480: 517-519.

Burke, C. M., J. Theodore, K. D. Dawkins. (1984). Post-transplant obliterative bronchiolitis and other late sequelae in human heart lung transplantation. *Chest* 86: 824-829.

Busschbach, J. J., P. E. Horikx, J. M. van-den-Bosch, A. Brutel-de-la-Riviere, and F. T. de-Charro. (1994). Measuring the quality of life before and after bilateral lung transplantation in patients with cystic fibrosis. *Chest* 105: 911-717.

Bussiéres, L. M., P. W. Pflugfelder, A. W. Taylor, E. G. Noble, and W. J. Kostuk. (1997). Changes in skeletal muscle morphology and biochemistry after cardiac transplantation. *Am. J. Cardiol.* 78: 630-634.

Cain D. F., A. A. Infante, and R. E. Davies. (1962). Chemistry of muscle contraction. *Nature* 196: 214-217.

Campbell, S. L., J. A. den Hollander, J. R. Alger, and R. G. Shulman. (1987). ^{31}P NMR saturation-transfer and ^{13}C NMR kinetic studies of glycolytic regulation during anaerobic and arobic glycolysis. *Biochemistry* 26: 7493-7500.

Capaldi, R. A. (1982). Arrangement of proteins in the inner mitochondrial membrane. *Biochem. Biophys. Acta* 694: 291-306.

Carere, R., G. A. Patterson, P. Lui, T. J Williams, J. R. Maurer, and R. F. Grossman. (1991). Right and left ventricular performance after double and single lung transpantation. *J. Thorac. Cardiovasc. Surg.* 102: 115-122.

Carini, R., M. Parola, M. U. Dianzani, and E. Albano. (1992). Mitochondrial damage and its role in causing hepatocyte injury during stimulation of lipid peroxidation by iron nitriloacetate. *Arch. Biochem. Biophysics* 297: 110-118.

Carroll, T. J., P. J. Abernethy, P. A. Logan, M. Barber, and M. T. McEniery. (1998). Resistance training frequency: strength and myosin heavy chain responses to two and three bouts per week. *Eur. J. Appl. Physiol.* 78: 270-275.

Cassina, A., and R. Radi. (1996). Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport. *Arch. Biochem. Biophys.* 328: 309-316.

Chance, B., and G. R. Williams. (1955). Respiratory enzymes in oxidative phosphorylation: III. the steady state. *J. Biol. Chem.* 16: 409-427.

Chance, B., and G. R. Williams. (1956). The respiratory chain and oxidative phosphorylation. *Adv. Enzymol.* 17: 65-134.

Chati, Z., F. Zannad, B. Robin-Lherbier, J. Escanye, C. Jeandel, J. Robert, and E. Aliot. (1994). Contribution of specific skeletal muscle metabolic abnormalities to limitation of exercise capacity in-patients with chronic heart failure: A phosphorus 31 unclear magnetic resonance study. *Am. Heart. J.* 128: 781-792.

Chati, Z., F. Zannad, C. Jeandel, B. Lherbier, J. M. Escanye, J. Robert, and E. Aliot. (1996). Physical deconditioning may be a mechanism for the skeletal muscle energy phosphate metabolism abnormalities in chronic heart failure. *Am. Heart J.* 131: 560-566.

Chia, L. S., J. E. Thompson, and M. A. Moscarello. (1983). Disorders in human myelin induced by superoxide radical: an in vitro investigation. *Biochem. Biophysiol. Res. Communi.* 117: 141-146.

Childs, G. V. (1998). Mitochondria. URL addrese: http://cellbio.utmb.edu/cellbio/mitochondria-l.htm.

Clamann, H. P., and K. T. Broecker. (1979). Relation between force and fatigability of red and pale skeletal muscle in man. *Am. J. Phys. Med.* 58: 70-85.

Coggan, A. R., R. J. Spina, D. S. King, M. A. Rogers, M. Brown, P. M. Nemeth, and J. O. Holloszy. (1992). Skeletal muscle adaptations to endurance training in 60- to 70-yr-old men and women. *J. Appl. Physiol.* 72: 1780-1786.

Cooke, R., K. Franks, G. B. Luciani, and E. Pate. (1988). The inhibition of rabbit skeletal muscle contraction by hydrogen ions and phosphate. *J. Physiol.* 395: 77-97.

Cooper, C. E, and G. C. Brown. (1995). The interactions between nitric oxide and brain nerve terminals as studied by electron paramagnetic resonance. *Biochem. Biophys. Res. Commun.* 212: 404-412.

Cooper, D. K., E. N. Samara, L. Mieles, H. Merhav, Imes-N, D. Van-Thiel, N. Zuhdi. (1994b). Survival following organ transplantation in an Oklahoma institution, 1985-1993. *J. Okla. State Med. Assoc.* 87: 506-508.

Cooper, J. D., G. A. Patterson, and E. P. Trulock. (1994a). Results of single and bilateral lung transplantation in 131 consecutive recipients. *J. Thorac. Cardiovasc. Surg.* 107: 460-470.

Cooper, J. D., M. Billingham, T. Egan, M. I. Hertz, T. Higenbottam, J. Lynch, J. Mauer, I. Paradis, G. A. Patterson, C. Smith, E. P. Trulock, C. Vreim and S. Yousem. (1993). A working formulation for the standardization of nomenclature and clinical staging of chronic dysfunction in lung allografts. *J Heart Lung transplant.* 12: 713-716.

Cooper, J. M., V. M. Mann, and A. H. Schapira. (1992). Analyses of mitochondrial respiratory chain function and mitochondrial DNA deletion in human skeletal muscle: effect of ageing. *J. Neurol. Sci.* 113: 91-98.

Cooper, R. R. and I. C. Iowa. (1972). Alterations during immobilization and regeneration of skeletal muscle in cats. *J. Bone. Joint. Surg.* 54: 919-951.

Corpier, C. L., P. H. Jone, W. N. Suki, E. D. Lederer, M. A. Quinones, S. W. Schmidt, and J. B. Young. (1988). Rhabdomyolysis and renal injury with lovastatin use: report of two cases in cardiac transplant recipients. *JAMA* 269: 239-241.

Cortopassi C. A., and N. Arnheim. (1990). Detection of a specific mitochondrial DNA deletion in tissues of older humans. *Nucleic Acids Res.* 18: 6927-6933.

Cortright, R. N., D. Zheng, J. P. jones, J. D. Fluckey, S. E. DiCarlo, D. Grujic, B. B. Lowell, and G. L. Dohm. (1999). Regulation of skeletal muscle UCP-2 and UCP-3 gene expression by exercise and denervation. *Am. J. Physiol.* 276: E217-E221.

Costill, D. L., E. F. Coyle, W. F. Fink, G. R. Lesmes, and F. A. Witzmann. (1979). Adaptations in skeletal muscle following strength training. *J. Appl. Physiol.* 46: 96-99.

Costill, D. L., J. Daniels, W. Evens, W. Fink, G. Krahenbuhl, and B. Saltin. (1976). Skeletal muscle enzymes and fiber composition in male and female track athletes. *J. Appl. Physiol.* 40: 149-154.

Costill, D., E. Coyle, G. Dalsky, W. Evans, W. Fink, and D. Hoopes. (1977). Effects of elevated plasma FFA and insulin on muscle glycogen usage during exercise. *J. Appl. Physiol.* 43: 695-699.

Côté, C., J. A. Simoneau, P. Lagasse, M. Boulay, M. C. Thibault, M. Marcotte, and C. Bouchard. (1988). Isokinetic strength training protocols: do they induce skeletal muscle fiber hypertrophy? *Arch. Phys. Med. Rehabil.* 69: 281-285.

Couetil, J. P., M. J. Tolan, D. F. Loulmet, A. Guinvarch, P. G. Chevalier, A. Achkar, P. Birmbaum, and A. F. Carpentier. (1997). Pulmonary bipartitioning and lobar transplantation: a new approach to donor organ shortage. *J. Thorac. Cardiovasc. Surg.* 113: 529-537.

Coyle, E. F., W. H. Martin III, D. R. Sinacore, M. J. Joyner, J. M. Hagberg, and J. O. Holloszy. (1984). Time course of less of adaptations after stopping prolonged intense endurance training. *J. Appl. Physol.* 57: 1854-1864.

Crapo, R. O., A. H. Morris, and R. M. Gardner. (1981). Reference spirometric values using techniques and equipment that meet ATS recommendations. *Am. Rev. Respir. Dis.* 123: 659-664.

Crapo, R. O., and A. H. Morris. (1981). Standardized single breath normal values for carbon monoxide diffusing capacity. *Am. Rev. Respir. Dis.* 123: 185-189.

Crompton, M. (1990). The role of Ca^{2+} in the function and dysfunction of heart mitochondria. In: *Calcium and the Heart*. Langer, G. A. (Ed.) Raven Press Ltd. New York. Pp.167-198.

Crompton, M., and A. Costi. (1988). Kinetic evidence for a heart mitochondrial pore activited by Ca^{2+}, inorganic phosphate and oxidative stress. *Eur. J. Biochem.* 178: 489-501.

Crompton, M., and A. Costi. (1990). A heart mitochondrial pore of possible relevance to reperfusion injury. *Biochem. J.* 266: 33-39.

Crompton, M., O. McGuinness, and W. Nazareth. (1992). The involvement of cyclosporin A binding in regulating and uncoupling mitochondrial energy transduction. *Biochem. Biophys. Aata* 1101: 214-217.

Cyert, M. (1992). Imminosuppressants hit the target. *Current Biol.* 2: 18-20.

Damerau, W., J. Ibel, T. Thürich, H. Assadnazari, and G. Zimmer. (1993). Generation of free radicals in Langendorff and working hearts during normoxia, hypoxia, and reoxygenation. *Basic. Res. Cardiol.* 88: 141-149.

Darley-Usmar, V. M., D. R. Smith, V. J. Oleary, D. Stone, D. J. Hardy and J. B. Clark. (1990). Hypoxia-reoxygenation induced damage in the myocardium: the role of mitochondria. *Biochem. Sco. Trans.* 18: 526-528.

Davies, K. J. A., A. T. Quintaniha, G. A. Brooks, and L. Packer. (1982). Free radical and tissue damage produced by exercise. *Biochem. Biophys. Res. Commun.* 107: 1198-1205.

Davies, K. J. A., L. Packer, and G. A. Brooks. (1981). Biochemical adaptation of mitochondria, muscle and whole-animal respiration to endurance training. *Arch. Biochem. Biophys.* 209: 539-554.

Davis, R. D., and M. K. Pasque. (1995). Pulmonary transplantation. *Ann. Surg.* 221: 14-28.

Dawkins, K. D., S. W Jamieson, S. A. Hunt, J. C. Baldwin, C. M. Burke, A. Morris, M. E. Billingham, J. Theodore, P. E. Oyer, E. B. Stinson, and N. E. Shumay. (1985). Long-time results and complications after combined heart and lung transplantation. *Circulation.* 71: 919-926.

De Groot, H., T. Noll, and H. Sies. (1985). Oxygen dependence and subcellular partitioning of hepatic menadione-mediated oxygen uptake. Studies with isolated hepatocytes, mitochondria, and microsomes from rat liver in an oxystat system. *Arch. Biochem. Biophys.* 243: 556-562.

De Hoyos, A., G. Patterson, J. R. Maurer, J. C. Ramirez, J. D. Miller, T. L. Winton, and The Toronto Lung Transplant Group. (1992). Pulmonary transplantation: early and late results. *J. Thora. Cardiovasc. Surg.* 103: 295-306.

De Meer, K., J. A. Jeneson, V. A. Gulmans, J. Vaan der Laag, and R. Berger. (1995). Efficiency of oxidative work performance of skeletal muscle in patients with cystic fibrosis. *Thorax*. 50: 980-983.

Dechecchi, M. C., E. Girella, G. Cabrini, and G. Berton. (1988). The km of NADH dehydrogenase is decreased in mitochondria of cystic fibrosis cells. *Enzyme*. 40: 45-50.

Decramer, M., V. de-Bock, and R. Dom. (1996). Functional and histologic picture of steroid-induced myopathy in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 153: 1958-1964.

Dekhuijzen, P. N. R., G. Gayan-Ramirez, A. Bisschop, V. D. Bock, R. Dom, and M. Decrame. (1995). Corticosteriod treatment and nutrititional deprivation cause a different pattern of atrophy in rat diaphragm. *J. Appl. Physiol.* 78: 629-637.

Dennis, C., N. Caine, L. Sharples, R. Smyth, T. Higenbottam, S. Stewart, T. Wreghitt, S. Large, F. C. Wells, and J. Wallwork. (1993). Heart-lung transplantation for end-stage respiratory disease in patients with cystic fibrosis at Papworth Hospital. *J. Heart Lung Transplant.* 12: 893-902

Denton, R. M. and A. P. Halestrap. (1979). Regulation of pyruvate metabolism in mammalian tissue. *Essays Biochem.* 15: 37-77.

Denton, R. M. and J. G. McCormack. (1985). Ca^{2+} transport by mammalian mitochondria and its role in hormone action. *Am. J. Physiol.* 249: E543-E554.

Denton, R. M., D. A. Richards, and J, G. Chin. (1978). Calcium ions and the regulation of NAD-link isocitrate dehydrogenase from mitochondria of heart and other tissues. *Biochem. J.* 176: 894-906.

Denton, R. M., P. J. Randle, and B. R. Martin. (1972). Stimulation by calcium ions of pyruvate dehydrogenase phosphatase. *Biochem. J.* 128: 161-163.

Depierre, J. W., and L. Ernster. (1977). Enzyme topology of intracellular membranes. *Annu. Rev. Biochem.* 46: 201-262.

Desplanches, D., M. H. Mayet, B. Sempore, J. Frutoso, and R. Flandrois. (1987). Structural and function responses to prolonged hindlimbs suspension in rat muscle. *J. Appl. Physiol.* 63: 558-563.

Dodd, S. T., S. K. Powers. E. Brooks, and M. P. Crawford. (1993). Effects of reduced O_2 delivery with anemia, hypoxia, or ischemia on peak $\dot{V}O_2$ and force in skeletal muscle. *J. Appl. Physiol.* 74: 186-191.

Dohm, G. L., H. Barakat, T. P. Stephenson, S. N. Pennington, and E. B. Tapscott. (1975). Changes in muscle mitochondrial lipid composition resulting from training and exhaustive exercise. *Life Sci.* 17: 1075-1080.

Dohm, G. L., R. L. Huston, E. W. Askew, and P. C. Weiser. (1972). Effects of exercise on activity of heart and muscle mitochondria. *Am. J. Physiol.* 223: 783-787.

Dons, B., K. Bollerup, F. Bonde-Petersen, and S. Hancke. (1979). The effect of weight-lifting exercise related to muscle fibre composition and muscle cross-sectional area in human. *Eur. J. Appl. Physiol.* 40: 95-106.

Dowling, R. D., M. Zenati, G. J. Burckart, S. A. Yousem, M. Schaper, R. L Simmons, R. L. Hardesty, and B. P. Griffith. (1990). Aerosolized cyclosporine as single-agent immunotherapy in canine lung allografts. *Surgery* 108: 198-204.

Draeger, A., A. G. Weeds, and R. B. Fitzsimons. (1987). Primary, secondary and tertiary myotubes in developing skeletal muscle: A new approach to the analysis of human myogenesis. *J. Neurolog. Sci.* 81: 19-43.

Drexler, H., U. Reide, T. Münzel, H. König, E. Funke, and H. Just. (1992). Alteration of skeletal muscle in chronic heart failure. *Circulation* 85: 1751-1759.

Dreyfuss, M., E. Harri, H. Hofmann, H. Kobel, W. Pache, and H. Tscherter. (1976). Cyclosporin A and C. New metabolites from Tricaderma polysporum (Link ex Pers.) Rifai. *Eur J Appl. Microl.* 3: 125-133.

Dubowitz, V., and G. Brookes. (1985). Muscle biopsy. In: *A Practical Approach.* 2nd edition. London: Bailliere Tindall.

Duchen, M., O. McGuinness, L. Brown, and M. Crompton. (1993). On the involvement of cyclospotin A sensitive mitochondrial pore in myocardial reperfusion injury. Cardiovasc. Res. 27, 1790-1794.

East, C., P. A. Alivizatos, S. M. Grundy, P. H. Jones, and J. A. Farmer. (1988). Rhabdomyolysis in patients receiving lovastatin after cardiac transplantation. *N. Engl. J. Med.* 318: 47-48.

Elimadi, A., D. Morin, R. Sapena, A. M. Chauvet-Monges, A. Crevat-A, and J. P. Tillement. (1997). Comparison of the effects of cyclosporine A and trimetazidine on Ca(2+)-dependent mitochondrial swelling. *Fundam. Clin. Pharmacol.* 11: 440-447

Ernster, L. and B. Kuylenstierna. (1970). Outer membrance of mitochondria, in *Membrances of mitochondria and chloroplasts*. E. Racker, (ed.), Van Nostrand Reinhold, New York, Pp. 172-212.

Essén, B. and J. Henriksson. (1980). Metabolic characteristics in human type 2 skeletal muscle fibers (Abstract). *Muscle Nerve* 3: 263.

Essén, B., E. Jansson, J. Henriksson, A. W. Taylor, and B Saltin. (1975). Metabolic characteristics of fibre types in human skeletal muscle. *Acta physiol. Scand.* 95: 153-165.

Essén-Gustavsson, B., and J. Henriksson. (1984). Ezyme levels in pools of microdissected human muscle fibres od identified type. Adaptive response to exercise. *Acta. Physiol. Scand.* 120: 505-515.

Essén-Gustavsson, B., and O. Borges. (1986). Histochemical and metabolic characteristics of human skeletal muscle in relation to age. *Acta Physiol. Scand.* 126:107-114.

Evans, A. B., A. J. Al-himyary, M. I. Hrovat, P. Pappagianopoulos, J. C. L. C. Wain Ginns, and D. M. Systrom. (1997). Abnormal skeletal muscle oxidative capacity after lung transplantation by ^{31}P-MRS. *Am. J. Respir. Crit. Care Med.* 155: 615-621.

Evans, W. (1997). Functional and metabolic consequences of sarcopenia. *J. Nutr.* 127 (Suppl): 998S-1003S.

Favero T. G., A. C. Zable, D. Colter, and J. J. Abramson. (1997). Lactate inhibits Ca(2+)-activated Ca(2+)-channel activity from skeletal muscle sarcoplasmic reticulum. *J Appl Physiol.* 82: 447-452.

Fawcett, D. W. (1994). Chapter 1 The cell. Mitochondria. In: *A textbook of histology.* Chappman and Hall. 12th edition. New York and London. Pp 22-26.

Febbraio, M. A., R. J. Snow, M. Hargreaves, C. S. Stathis, I. K. Martin, and M. F. Carey. (1994). Muscle metabolism during exercise and heat stress in trained men: effect of acclimation. *J. Appl. Physiol.* 76: 589-597.

Fehér, J., G. Csomós and A. Vereckei. (1987). Free radical reactions in medicine. Springer-Verlag Berlin Heidelberg press. Germany. Pp. 9.

Feigenhaum, M. S., and M. L. Pollock. (1999). Prescription of resistance training for health and disease. *Med. Sci. Sports Exerc.* 31: 38-45.

Felig, P. (1975). Amino acid metabolism in man. *Ann. Rev. Biochem.* 44: 993-955.

Fell, R. D., J. M. Steffen, and X. J. Musacchia. (1985). Effect of hypokinesia-hypodynamia on rat muscle oxidative capacity and glucose uptake. *Am. J. Physiol.* 249: R308-R312.

Ferketich, A. K., T. E. Kirby, and S. E. Alway. (1998). Cardiovascular and muscular adaptation to combined endurance and strength training in elderly women. *Acta Physiol. Scand.* 164: 259-267.

Fernandez-Sola, J., J. Campistol, J. Casademont, J. M. Grau, and A. Urbanno-Marquez. (1990). Reversible cyclosporin myopathy. *Lancet* 335: 362-363.

Ferrando, A. A., K. D. Tipton, M. M. Bamman, and R. R. Wolfe. (1997). Resistance exercise maintains skeletal muscle protein synthesis during bed rest. *J. Appl. Physiol.* 82: 807-810.

Ferrari, R. (1996). The role of mitochondria in ischaemic heart disease. *J. Cardiaovasc. Pharmacol.* 28 (suppl.1): S1-S10.

Ferretti, G., G. Antonutto, C. Denis, H. Hoppeler, A. E. Minetti M. V. Narici, and D. Desplanches. (1997). The interplay of central and peripheral factors in limiting maximal O2 consumption in man after prolonged bed rest. *J. Physiol.* 501: 677-686.

Fiaccadorie, E., S. Del Canale, P. Vitali, E. Coffrini, N. Ronda, and A. Guariglia. (1987). Skeletal muscle energetics, acid-base equilibrum and lactate metabolism in patients with severe hypercapnia and hypoxia. *Chest* 92: 883-887.

Fielding, R. A. (1995). The role of progressive resistance training and nutrition in the preservation of lean body mass in the elderly. *J. Am. Coll. Nutr.* 14: 587-594.

Fitts, R. H. (1994). Cellular mechanisms of muscle fatigue. *Physiol. Rev.* 74: 49-93.

Fleury, C., M. Neverova, S. Collins, S. Raimbault, O. Champigny, C. Levi-Meyrueis, F. Bouillaud, M. F. Seldin, S. R. urwit, D. Ricquier, and C. H. Warden. (1997). Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nat. Genet.* 15: 269-272.

Fontaine, E., O. Eriksson, F. Ichas, and P. Bernardi. (1998). Regulation of the permeability transition pore in skeletal muscle mitochondria. *J. Bio. Chem.* 273: 12662-12668.

Fournier, N., G. Ducet, and A. Crevat. (1987). Action of cyclosporine on mitochondrial calcium fluxes. *J. Bioenerg. Biomembr.* 19: 297-303.

Fraga, C. G., M. K. Shigenaga, J. W. Park, P. Degan, and B. N. Ames. (1990). Oxidative damage to DNA during aging: 8-hydroxy-2'-deoxyguanosine in rat organ DNA and urine. *Proc. Natl. Acad. Sci.* 87: 4533-4537.

Fragomeni, L. S., and M. P. Kaye. (1988). The registry of the international society for heart transplantation: Fifth of official report—1988. *J. Heart Transplant* 7: 249-253.

Frei, B., M. C. Kim, and B. N. Ames. (1990). Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. *Proce. National Acad. Sci.* USA, 87: 4879-4883.

Frerman, F. E., and S. I. Goodman. (1985). Fluorometric assay of acyl-CoA dehydrogenases in normal and mutant human fibroblasts. *Biochem. Med.* 33: 38-44.

Frist, W. H., C. H. Lorenz, E. S. Walker, J. E. Loyd, J. R. Stewart, T. P. Jr. Graham, D. P. Pearlstein, S. P. Key, and W. H. Merrill. (1995). MRI complements standard assessment of right ventricular function after lung transplantation. *Ann. Thorac. Surg.* 60: 268-271.

Fry, A. C., C. A. Allemeier, and R. S. Staron. (1994). Correlation between percentage fiber type area and myosin heavy chain content in human skeletal muscle. *Eur. J. Appl. Physiol.* 68: 246-251.

Gaitanos, G. C., C. Williams, L. H. Boobis, and S. Brooks. (1993). Human muscle metabolism during intermittent maximal exercise. *J. Appl. Physiol.* 75: 712-719.

Gandevia, B., and P. Hugh-Jones. (1957). Terminology for measurements of ventilatory capacity. *Thorax* 1: 290-293.

Gardiner, P. F., M. Favron, and P. Corriveau. (1992). Histochemical and contractile responses of rat medial gastrocnemius to 2 weeks of complete disuse. *Can. J. Physiol. Pharmacol.* 70: 1075-1081.

Garnett, R. A. F., M. J. O'Donovan, J. A. Stephens, and A. Tayior. (1978). Motor unit organization of human medical gastrocnemius. *J. Physiol.* 287: 33-43.

Gellerfors, P., and M. Linden. (1981). Biogenesis of the outer mitochondrial membrane in isolated rat hepatocytes. *FEBS Lett.* 5,127: 91-93.

Gettman, L. R., J. J. Ayres, M. L. Pollock, J. L. Durstine, and W. Grantham. (1979). Physiologic effects on adult men of circuit strength training and jogging. *Arch. Phys. Med. Rehabil.* 60: 115-120.

Gollnick, P. D. (1986). Metabolic regulation in skeletal muscle: influence of endurance training as exerted by mitochondrial protein concentration. *Acta Physiol. Scand.* (Suppl) 556: 56-66.

Gollnick, P. D., and D. W. King. (1969). Effect of exercised training on mitochondria of rat skeletal muscle. *Am. J. Physiol.* 216: 1502-1509.

Gollnick, P. D., L. A. Bertocci, T. B. Kelso, E. H. Witt, and D. R. Hodgson. (1990). The effect of high-intensity exercise on the respiratory capacity of skeletal muscle. *Pflügers. Arch.* 415: 407-413.

Goodman, M. N., and J. M. Lowenstein. (1977). The purine nucleotide cycle. Studies of ammonia production by skeletal muscle in situ and in perfused preparations. *J. Biol. Chem.* 252: 5054-5060.

Goreham, C., H. J. Green, M. Ball-Burnett, and D. Ranney. (1999). High-resistance training and muscle metabolism during prolonged exercise. *Am. J. Physiol.* 276: E489-E496.

Gosselink, R., and M. Decramer. (1998). Peripheral skeletal muscles and exercise performance in patients with chronic obstructive pulmonary disease. *Monaldi. Arch. Chest. Dis.* 1998 53: 419-423.

Goy, J. J., S. C. Stauffer, J. P. Dernaqz, D. Gillard, U. Kaufmann, T. Kuntzer, and L. Kappenherger. (1989). Myopathy as a possible side-effect of cyclosporin. *Lancet* I: 1446-1447.

Green H. J., I. G. Fraser, and D. A. Ranney. (1984). Male and female differences in enzyme activities of energy metabolism in vastus lateralis muscle. *J. Neurol. Sci.* 65: 323-331.

Green, D. E., and A. Tzagoloff. (1966). The mitochondrial ETC. *Arch. Biochem. Biophys.* 116: 293-304.

Green, D. E., W. F. Loomis, and V. H. Auerbach. (1948). Studies on the cyclophorase system. I. The complete oxidation of pyruvate acid to carbon dioxide and water. *J. Biol. Chem.* 172: 389-

Green, H. J. (1998). Cation pumps in skeletal muscle potential role in muscle fatigue. *Acta physiolo. Scand.* 201-213.

Green, H. J., A. Dahly, K. Shoemaker, C. Goreham, E. Bombardier, and M. Ball-Burnett. (1999). Serial effcts of high-resistance and prolonged endurance training on Na^+-

K^+ pump concentration and enzymatic activities in human vastus lateralis. *Acta Physiol. Scand.* 165: 177-184.

Green, H. J., C. Goreham, J. Ouyang, M. Ball-Burnett, and D. Ranney. (1998). Regulation of fiber size, oxidative potential, and capillarization in human muscle by resistance exercise. *Am. J. Physiol.* 276: R591-R596.

Green, H. J., R. Helyar, M. Ball-Burnett, N. Kowalchuk, S. Symon, and B. Farrance. (1992). Metabolic adaptations to training precede changes in muscle mitochondrial capacity. *J. Appl. Physiol.* 72: 484-491.

Greenhaff, P. L., J. M. Ren, K. SöDerlund, and E. Hultman. (1991). Energy metabolism in single human muscle fibres during contraction without and with epinephrine infusion. *Am. J. Physiol.* 260: E713-E718.

Griffiths, E. J., and A. P. Halestrap. (1993). Protection by cyclosporine A of ischemia/reperfusion-induced damage in isolated rat hearts. *J. Mol. Cell Cardiol.* 25: 1461-1469.

Groot, P. H. E., H. R. Scholte, and W. C. Hülsmann. (1976). Fatty acid activation: specificity, localization, and function. In: *Advances in lipd research.* Paoletti, R. and D. Krichevsky. (eds.), Academic press, New York. 14, Pp. 75-126.

Gross, C. R., K. Savik, R. M. Bolman, and M. I. Hertz. (1995). Long-term health status and quality of life outcomes of lung transplant recipients. *Chest* 108: 1578-1593.

Grossman, R. F., A. Frost, N. Zamel, G. A. Patterson, J. D. Cooper, P. R. Myron, C. L. Dear, and J. Maurer. (1990). Results of single lung transplantation for bilateral pulmonary fibrosis. *N. Eng. J. Med.* 322: 727-733.

Gülch, R. W. (1994). Force-volocity relations in human skelatal muscle. *Int. J. Sports Med.* 15 S2-S10.

Gunter, T. E., and D. R. Pfeiffer. (1990). Mechanisms by which mitochondria transport calcium. *Am. J. Physiol.* 258: C755-C786.

Häggmark, T., E. Jansson, and E. Eriksson. (1981). Fiber type area and metabolic potential of the thigh muscle in man after knee surgery and immobilization. *Int. J. Sports Med.* 2: 12-17.

Hakkinen, K., M. Alen, and P. V. Komi. (1985). Changes in isometric force- and relaxation-time, electromyographic and muscle fibre characteristics of human skeletal muscle during strength training and detraining. *Acta Physiol. Scand.* 125: 573-585

Hakkinen, K., M. Kallinen, M. Izquierdo, K. Jokelainen, M. Lassila, E. Malkia, W, J, Kraemer, R. U. Newton, and M. Alen. (1998) Changes in agonist-antagonist EMG, muscle CSA, and force during strength training in middle-aged and older people. *J Appl. Physiol.* 84: 1341-1349.

Halestrap, A. P. (1994). Chapter 5. Interactions between oxidative stress and calcium overload on mitochodnrial function. In: *Mitochondrial DNA, protein, and disease.* Darley-Usmar, V., and A. H. V. Schapira (eds.). Portland, London. Pp. 130.

Halkjær-Kristensen, J., and T. Ingemann-Hansen. (1985a). Wasting of the human quadriceps muscle after knee ligament injuries. II. Muscle fibre morphology. *Scand. J. Rehab. Med. (Suppl)* 13: 12-20.

Halkjær-Kristensen, J., and T. Ingemann-Hansen. (1985b). Wasting of the human quadriceps muscle after knee ligament injuries. III. Oxidative and glycolytic enzyme activities. *Scand. J. Rehab. Med. (Suppl)* 13: 21-28.

Halkjær-Kristensen, J., and T. Ingemann-Hansen. (1985c). Wasting of the human quadriceps muscle after knee ligament injuries. IV. Dynamic and static muscle function. *Scand. J. Rehab. Med. (Suppl)* 13: 29-37.

Hambrecht, R., E. Fiehn, J. Yu, J. Niebauer, C. Weigl, L. Hilbrich, V. Adams, U. Riede, and G. Schuler. (1997). Effects of endurance training on mitochondrial ultrastructure and fiber type distribution in skeletal muscle of patients with stable chronic heart failure. *J. Am. Coll. Cardiol.* 29: 1067-1073.

Hamilton, A. L., K. J. Killian, E. Summers, and N. L. Jones. (1995). Muscle strength, symptom intensity, and exercise capacity in patients with cardiorepiratory disorders. *Am. J. Respir. Crit. Care Med.* 152: 2021-2031.

Hansford, R. G., and J. B. Chappell. (1967). The effect of Ca2+ on the oxidation of glycerol phosphate by blowfly flight-muscle mitochondria. *Biochem. Biophys. Res. Commun.* 27: 686-692.

Hanson, P., A. Dive, J. M. Brucher, M. Bisteau, M. Dangoisse, and T. Deltombe. (1997). Acute corticosteroid myopathy in intensive care patients. *Muscle & Nerve* 20: 1371-1380.

Hargreaves, M., J. P. Finn, R. T. Withers, J. A. Halbert, G. C. Scroop, M. Mackay, R. J. Snow, and M. F. Carey. (1997). Effect of muscle glycogen availability on maximal exercise performance. *Eur. J. Appl. Physiol.* 75: 188-192.

Harper, M. E., S. Monemdjou, J. J. Ramsey, and R. Weindruch. (1998). Age-related increase in mitochondrial proton leak and decrease in ATP turnover reactions in mouse hepatocytes. *Am. J. Physiol.* 1998 275: E197-E206.

Harris, R. C., E. Hulman, and L. O. Nordesjö. (1974). Glycogen, glycolytic intermediates and hight energy phosphates determined in biopsy samples of values. *Scan. J. Clin. Lab. Invest.* 33: 109-120.

Hartl, F. U., N. Pfanner, D. W. Nicholson, and W. Neupert. (1988). Mitochondrial protein import. *Biochem. Biophys. Acta.* 988: 1-45.

Hasselbach, W. (1964). Relaxing factorory and relaxation of muscle. *Prog. Biophys. Mol. Biol.* 14: 167-222.

Hasselgren P. O., and J. E. Fischer. (1997). The ubiquitin-proteasome pathway: Review of a novel intracellular mechanism of muscle protein breakdown during sepsis and other catabolic conditions. *Ann. Surg.* 225: 307-316.

Hasselgren P. O., J. H. James, and J. E. Fischer. (1986). Inhibited muscle amino acid uptake in sepsis. *Ann. Surg.* 203: 360-365.

Hasselgren P. O., J. H. James, D. W. Benson, M. Hallangeras, U. Angeras, D. T. Hiyama, S. Li, and J. E. Fischer. (1989). Total and myofibrillar protein breakdown in different types of rat skeletal muscle: effects of sepsis and regulation by insulin. *Metabolism* 38: 634-640.

Hatefi, Y. (1985). The mitochondrial electron transport and oxidative phosphorylation system. *Ann. Rev. Biochem.* 54: 1015-1019.

Hatefi, Y., A. G. Haavik, and D. E. Griffiths. (1962a). Studies of the electron transfer system. XL. Preparation and properties of mitochondrial DPNH-coenzyme Q reductase: *J. Biol. Chem.* 237: 1676-1680.

Hatefi, Y., A. G. Haavik, and D. E. Griffiths. (1962b). Studies of the electron transfer system. XLI. Reduced coenzyme Q-cytochrome c reductase. *J. Biol. Chem.* 237: 1681-1685.

Hatefi, Y., A. G. Haavik, R. Fowler, and D. E. Griffiths. (1962c). Studies of the electron transfer system. XLII. Reconstitution of the elctron transfer system. *J. Biol. Chem.* 237: 2661-2669.

Hatefi, Y., and Y. M. Galante. (1980). Isolation of cytochrome b560 from Complex II (succinateubiqnone oxidoreductase) and its reconstitution with succinate dehydrogenase. *J. Biol. Chem.* 255: 5530-5537.

Hather, B. M., P. A. Tesch, P. Buchanan, and G. A. Dudley. (1991). Influence of eccentric actions on skeletal muscle adaptations to resistance training. *Acta Physiol. Scand.* 143: 177-185.

Haworth, R. A., and D. R. Hunter. (1980). Allosteric inhibition of the Ca2+ activated hydrophilic channel of the mitochondrial inner membrane by nucleotides. *J. Membr. Biol.* 54: 231-236.

Hayakawa, M., K. Torii, S. Sugiyama, M. Tanaka, and T. Ozawa. (1991). Age-associated accumulation of 8-hydroxydeoxyganosine in mitochondrial DNA of human diaphragm. *Biochem. Biophys. Res. Commun.* 179: 1023-1029.

Hellsten-Westing, Y., L. Kaijser, B. Ekblom, and B. Sjödin. (1994). Exchange of purines in human liver and skeletal muscle with short-term exchaustive exercise. *Am. J. Physiol.* 266: R81-R86.

Henrich, H. A., W. Romen, W. Heimgärtner, E. Hartung, and F. Bäumer. (1988). Capillary rarefaction characteristic of the skeletal muscle of hypertensive patients. *Klin Wochenschr.* 66: 54-60.

Henriksson, J. (1992). Effects of physical training on the metabolism of skeletal muscle. *Diabetes Care.* 15: 1701-1712.

Hepple, R. T., S. L. M. Mackinnon, S. G. Thomas, J. M. Goodman, and M. J. Plyley. (1997). Quantitating the capillary supply and the response to resistance training in older men. *Pflugers Arch.* 433: 238-244.

Hermansen, L., and J. Osnes. (1972). Blood and muscle pH after maximal exercise in man. *J. Appl. Physiol.* 32: 304-308.

Herrick, K. R., D. Salazar, S. I. Goodman, G. Finocchiaro, L. A. Bedzyk, and F. E. Frerman. (1994). Expression and characterization of human and chimeric human-Paracoccus denitrificans electron transfer flavoproteins. *J. Biol. Chem.* 269: 32239-32245.

Hickson, R. C., B. A. Dvorak, E. M. Gorostiaga, T. T. Kurowski, and C. Foster. (1988). Potential for strength and endurance training to amplify endurance performance. *J. Appl. Physiol.* 65: 2285-2290.

Hickson, R. C., M. A. Rosenkoetter, and M. M. Brown. (1980). Strength training effects on aerobic and short-term endurance. *Med. Sci. Sports Exerc.* 12:336-339.

Hildebrand, I. L., C. Sylvén, M. Esbjornsson, K. Hellstrom, and E. Jansson. (1991). Does hypoxaemia induce transformations of fibre types? *Acta Physiol. Scand.* 141: 435-439.

Hirvonen, J., S. Rehunen, H. Rusko, and Härkönen. (1987). Breakdown of high-energy phosphate compounds and lactate accumulation during short supramaximal exercise. *Eur. J. Appl. Physiol.* 56: 253-259.

Hofhaus, G., H. Weiss, and K. Leonard. (1991). Electron microscopic analysis of the peripheral and membrane part of mitochonsrial NADH dehydrogenase (Complex I). *J. Mol. Biol.* 221: 1027-1043.

Hokanson, J. F., J. G. Mercier, and G. A. Brooks. (1995). Cyclosporine A decreases rat skeletal muscle mitochondrial respiration in vitro. *Am. J. Respir. Crit Care Med.* 151: 1848-1851.

Holloszy, J. O. (1967). Biochemical adaptations in muscle. *J. Bio. Chem.* 242: 2278-2282.

Holloszy, J. O. (1976). Adaptations of muscular tissue to training. *Prog. Cardiovasc. Dis.* 18: 445-458.

Holloszy, J. O., and E. F. Coyle. (1984). Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J. Appl. Physiol.* 56: 831-838.

Holloszy, J. O., L. B. Oscal, I. J. Don, and P. A. Mole. (1970). Mitochondrial citric acid cycle and related enzymes, adaptive response to exercise. *Biochem. Biophy. Rse. Commun.* 40: 1368-1373.

Holt, I. J., A. E. Harding, J. M. Cooper, A. H. V. Schapira, A. Toscano, J. A. Morgan-Hughes, and J. B. Clark. (1989). Mitochondrial myopathies clinical and biochemical features of 30 patients with major deletions of muscle mitochondrial DNA. *Ann. Neurol.* 26: 699-708.

Hood, D. A., and R. I. Terjung. (1990). Amino acid metabolism during exercise and following endurance training. *Sports Med.* 9: 23-35.

Hoppeler, H., P. Lüthi, H. Claassen, E.R. Weibel, and H. Howald. (1973). The ultrastructure of the normal human skeletal muscle: A morphometric analysis on untrained men, women and well-trained orienteers. *Pflügers Arch.* 344: 217-232.

Hosenpud, J. D., L. E. Bennett, B. M. Keck, B. Fiol, and R. J. Novick. (1997). The Registry of the International society for Heart and Lung Transplantation: Fourteenth Official Report-1997. *J. Heart Lung Transplant* 16: 691-712.

Housh, D. J., and T. J. Housh. (1993). The effects of unilateral velocity-specific concentric strength training. *J. Orthop. Sports Phys. Ther.* 17: 252-256.

Housh, D. J., T. J. Housh, J. P. Weir, L. L. Weir, G. O. Johnson, and J. R. Stout. (1995). Anthropometric estimation of thigh muscle cross-sectional area. *Med. Sci. Sports Exerc.* 27: 784-791.

Houston, M. E., E. A. Froese, St.P. Valeriote, H. J. Green, and D. A. Ranney. (1983). Muscle performance, morphology and metabolic capacity during strength training and detraining: A one leg model. *Eur J. Appl. Physiol.* 51: 25-35.

Howald, H., H. Hoppeler, H. Classen, O. Mathieu, and R. Straub. (1985). Influences of endurance training on the ultrastructural composition of the different muscle fibre types in humans. *Pflügers Arch.* 403: 369-376.

Howard, D. K., E. Iademarco, and E. P. Trulock. (1994). The role of cardiopulmonary exercise testing in lung and heart-lung transplantation. *Cli. Chest Med.* 15: 405-420.

Hultman, E. (1967). Studies on muscle metabolism of glycogen and active phosphate in man with special reference to exercise and diet. *Scand. J. Clin. Lab. Invest.*19 (Suppl) 94: 1-63.

Hunter, D. R., and R. A. Haworth. (1979). The Ca2+ induced membrane transition in mitochondria. I. The protective mechanisms. *Arch. Biochem. Biophys.* 195: 453-459.

Hurley, B. F., P. M. Nemeth, W. H. Martin III, J. M. Hagberg, G. P. Dalsky, and J. O. Holloszy. (1986). Muscle triglyceride utilization during exercise: effect of training. *J Appl. Physiol.* 60: 562-567.

Imms, F. J., A. J. Hackett, S. P. Prestidge and R. H. Fox. (1977). Voluntary isometric muscle strength of patients undergoing rehabilitation following fractures of the lower limb. *Rheuma.& Rehabi.* 16: 162-171.

Ingemann-Hansen, T., and J. Halkjaer-Kristensen. (1985a). Physical training of the hypotrophic quadriceps muscle in man. I. The effects of different training programs on muscle performance. *Scand. J. Rehab. Med. (supple).* 13: 38-44.

Ingemann-Hansen, T., and J. Halkjaer-Kristensen. (1985b). Physical training of the hypotrophic quadriceps muscle in man. II. The effcts on lean and fat conponent and muscular performance. *Scand. J. Rehab. Med. (supple).* 13: 45-49.

Ingemann-Hansen, T., and J. Halkjaer-Kristensen. (1985b). Physical training of the hypotrophic quadriceps muscle in man. III. The effects on morphology and enzymatic capacity. *Scand. J. Rehab. Med. (supple).* 13: 50-55.

Ingjer, F. (1979). Effects of endurance training on muscle fibre ATP-ase activity, capillary supply and mitochondrial content in man. *J. Physiol.* 294: 419-432.

Jackman, M. R., and W. T. Willis. (1996). Characteristics of mitochondria isolated from type I and type Iib skeletal muscle. *Am. J. Physiol.* 270: C673-C678.

Jackson, A. S., and M. L. Pollock. (1978). Generalised equations for predicting body density in men. *Br. J. Nutr.* 40, 497-504.

Jackson, A. S., M. L Pollock, and A. Ward. (1980). Generalised equations for predicting body density of women. *Med. Sci. Sports Exerc.* 12: 175-504.

Jakobsson, P., and L. Jorfeldt. (1995). Long-term oxygen therapy may improve skeletal muscle metabolism in advanced chronic obstractive pulmonary disease patients with chronic hypoxaemia. *Respir. Med.* 89: 471-476.

Jakobsson, P., L. Jorfeldt, and A. Brundin. (1990). Skeletal muscle metabolism and fibre types in patients with advance chronic obstuctive pulmonary disease (COPD), with and without chronic respiratory failure. *Eur. Respir. J.* 3: 192-196.

Jakobsson, P., L. Jorfeldt, and J. Henriksson. (1995). Metabolic enzyme activity in the quadriceps femoris muscle in patients with severe chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 151: 374-377.

Janero, D. R. (1990). Malondialdehyde and thiobarbituric acid-ractivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radic. Biol. Med.* 9: 515-540.

Jänkälä, H., V. P. Harjola, N. E. Petersen, and M. Härkönen. (1997). Myosin heavy chain mRNA transform to faster isoforms in immobilized skeletal muscle: a quantitative PCR study. *J. Appl. Physiol.* 82: 977-982.

Jansson E., C. Sylvévidsson and E. Eriksson. (1988). Increase in myoglobin content and decrease in oxidative enzyme activities by leg muscle immobilization in man. *Acta Physiol. Scand.* 132: 515-517.

Jansson, E., G. A. Dudley, B. Norman, and P. Tesch. (1987). ATP and IMP in single human muscle fibers after high intensity exercise. *Clin. Physiol.* 7: 337-345.

Jenkins, R. R. (1988). Free radicals chemistry. *Sports Med.* 5: 156-170.

Jensen, R. L., F. G. Yanowitz and R. O. Crapo. (1991). Exercise hemodynamics and oxygen delivery measurement in heart transplant patients. *Am. J. Cardiol.* 68: 129-133.

Ji, L. L. (1995). Chapter 5. Exercise and oxidative stress: role of the cellular antioxidant systems. *Ex. sports Sci. Rev.* Pp. 135-166.

Jones, D. A., and O. M. Rutherford. (1987). Human muscle strength training: the effects of three different regimes and the nature of the resultant changes. *J. Physiol.* 391: 1-11.

Jones, N. L. (1997). Interpretation of stage 1 exercise test results. In: *Clinical Exercise Testing*. N. L. Jones (ed.). 4th edition. W.B. Saunders Company, Philadelphia, USA. Pp. 124-149.

Jones, N. L. L. Makrides, C. Hitchcock, T. chypchar, and N. McCartney. (1985). Normal standards for an incremental progressive cycle ergometer test. *Am. Resp. Respir. Dis.* 131: 700-708.

Juhlin-Dannfelt, A., M. Frisk-Holmberg, J. Karlsson, and P. Tesch. (1979). Central and peripheral circulation in relation to muscle-fibre composition in normo-and hyper-tensive man. *Clin. Sci.* 56: 335-340.

Jung, K. and C. Reinholdt. (1987). Inhibitory effect of cyclosporine on the respiratory efficiency of isolated human kidney mitochondria. *Transplantation* 43: 162-163.

Jung, K., and M. Pergande. (1985). Influence of cyclosporine A on the respiratory of isolated rat kidney mitochondria. *FEBS.* 2447 183: 167-169.

Jurimae, J., P. J. Abernethy, K. Blake, and M. T. McEniery. (1996). Changes in the myosin heavy chain isoform profile of the triceps brachii muscle following 12 weeks of resistance training. *Eur. J. Appl. Physiol.* 74: 287-292.

Kagan, V., E. Serbinova, and L. Packer. (1990). Antioxidant effects of ubiquinones in microsomes and mitochondria are mediated by tocopherol recycling. *Biochem. biophysical. Res. Commun.* 169: 851-857.

Kahan, B. D. (1987). Immunosuppressive therapy with cyclosporine for cardiac transplantation. *Circulation* 75: 40-56.

Kanehisa, H., S. Ikegawa, and T. Fukunaga. (1994). Comparison of muscle cross-sectional area and strength between untrained women and men. *Eur. J. Appl. Physiol.* 68: 148-154.

Karlsson, J. (1997). Chapter 3. Principles of radical formation. In: *Antioxidants and exercise*. Champaign, IL: Human Kinetics press, USA. Pp.25-37.

Karlsson, J., and B. Saltin. (1970). Lactate, ATP and CP in working muscles during exhaustive exercise in man. *J. Appl. Physiol.* 29: 598-602.

Kasch, F. W. (1976). The effects of exercise on the aging process. *Physiol. Sports Med.* 4: 64-69.

Katz, A., and K. Sahlin. (1990). Role of oxygen in regulation of glycolysis and lactate production in human skeletal muscle. *Exer. Sports Sci. Rev.* 18: 1-28.

Kauhanen, S., I. Leivo, and J. E. Michelsson. (1993). Early muscle changes after immobilization. An experimental study on muscle damage. *Clin. Orthop.* 297: 44-50.

Kavanagh, T. K., M. H. Yacoub, D. J. Mertens, J. Kennedy, R. B. Campell, and P. Sawyer. (1988). Cardiorespiratory responses to exercise training after orthotopic heart transplantation. *Circulation* 77: 162-171.

Kawasaki, S., S. Sugiyama, N. Ishiguro, T. Ozawa, and T. Miura. (1993). Implication of superoxide radicals on ischemia-reperfusion-induced skeletal muscle injury in rats. *Eur. Surg. Res.* 25: 129-136.

Kayanakis, J. G. (1989). The syndrome of "muscle deconditioning" in chronic insufficiency. *Arch. Mal. Corur. Vaiss.* 82: 1455-1458.

Keown, P. A., C. R. Stiller, A. L. Laupacis, W. Howson, R. Coles, M. Stawecki, J. Koegler, G. Carruthers, N. McKenzie, and N. R. Sinclair. (1982). The effects and side effects of cyclosporine: relationship to drug pharmacokinetics. *Transplant Proc.* 14: 659-661.

Kimoff, R. J., T. H. Cheong, M. G. Cosio, A. Guerrty, and R. D. Levy. (1990). Pulmonary denervation in humans. *Am. Rev. Respir. Dis.* 142: 1034-1040.

Kingsley-Hickman, P. B., E. Y. Sako, P. Mohanakrishnan, P. M. L. Robitaille, A. H. L. From, J. E. Foker, and K. U□urbil. (1987). ^{31}P NMR studies of ATP synthesis and hydrolysis kinetics in the intact myocardium. *Biochemistry* 26: 7501-7510.

Kirkwood , S. P., Munn, E. A., and G. A. Brooks. (1986). Mitochondrial reticulum in limb skeletal muscle. *Am. J. Physol.* 251: C395-C402.

Komi, P. V., J. T. Viitasalo, R. Rauramaa, and V. Vihko. (1978). Effect of isometric strength training on mechanical, electrical, and metabolic aspects of muscle function. *Eur. J. Appl. Physiol.* 40: 45-55.

Korzeniewski, B. (1998). Regulation of ATP supply during muscle contraction: theoretical studies. *Biochem. J.* 330: 1189-1195.

Kraemer, W. J., J. F. Patton, S. E. Gordon, E. A. Haman, M. R. Deschenes, K. Reynolds, R. U. Newton, N. T. Triplett, and J. E. Dziados. (1995). Compatibility of high-intensity strength and endurance training on hormonal and skeletal muscle adaptation. *J. Appl. Physiol.* 78: 976-989.

Kramer, M. R., H. A. Valantine, S. E. Marshall, V. A. Starnes, and J. Theodore. (1994). Recovery of the right ventricle after single-lung transplantation in pulmonary hypertension. *Am. J. Cardiol.* 73: 494-500.

Kramer, M. R., J. A. Smith, P. M. McCarthy, G. J. Berry, S. E. Marshall, J. Theodore, and N. J. Lewiston. (1996). Later complication and results in heart-lung and lung transplantation and future directions in cardiopulmonary transplantation. In: *The Stanford manual of cardiopulmonary transplantation.* J. A. Smith, P. M. McCarthy, G. E. Sarris, E. B. Stinson, and B. A. Reitz (Eds.). Futura publishing Co., Inc. Armonk, New York, Pp 263-291.

Krebs, H. A. and W. A. Johnson. (1937). The role of citric acid in intermediary metabolism in animal tissue. *Enzymologia* 4: 148.

Krieger, D.A., C.A. Tate, J. McMillin-Wood, and F.W. Booth. (1980). Populations of rat skeletal muscle mitochondria after exercise and immobilization. *J. Appl. Physiol.* 48: 23-28.

Kroshus, T. J., V. R. Kshettry, K. Savik, R. John, M. I. Hertz, and R. M. 3rd Bolman. (1997). Risk factors for the development of brinchiolitis obliterans syndrome after lung transplantation. *J. Thorac. Cardiovasc. Surg.* 114: 195-202.

Krysztopik, R. J., F. R. Bentley, D. A. Spain, M. A. Wilson, and R. N. Garrison. (1997). Lazaroids prevent acute cyclosporine-induced renal vasoconstriction. *Transplantation* 63: 1215-1220.

Kshettry, V. R., T. J. Kroshus, K. Savik, M. I. Hertz, and R. M. Bolman. (1996). Primary pulmonary hypertension as a risk factors for the development of obliterative bronchiolitis in lung allograft recioients. *Chest* 110: 704-709.

Kutsuzawa, T., S. Shioya, D. Kurita, M. Haida, Y. Ohta, and H. Yamabayshi. (1992). ^{31}P-NMR study of skeletal muscle metabolism in patients with chronic respiratory impairment. *Am. Rev. Respir. Dis.* 146: 1019-1024.

Kutsuzawa, T., S. Shioya, D. Kurita, M. Haida, Y. Ohta, and H. Yamabayshi. (1995). Muscle energy metabolism and nutritional statue in patients with chronic obstructive

pulmonary disease. A 31P magnetic resonance study. *Am. J. Respir. Crit. Care Med.* 152: 647-652.

L'Azou, B., B. Lakhdar, and J. Cambar. (1994). Use of two in vitro glomerular models to study the renal vasoreactivity of cyclosporine. *Transplant. Proc.* 26: 2883-2885.

Larner A. J., S. G. Sturman, J. B. Hawkins and M. Anderson. (1994). Myopathy with ragged red fibers following renal transplantation: possible role of cyclosporin-reduced hypomagnesaemia. *Acta Neuropathol.* 88: 189-192.

Lee, C. P., Q. Gu, Y. Xiong, R. A. Mitchell, and I. Ernster. (1996). P/O ratios reassessed: mitochondrial P/O ratio consistantly exceed 1.5 with succinate and 2.5 with NAD-linked substrates. *FASEB J.* 10: 345-350.

Lehninger, A. L., D. L. Nelson, and M. M. Cox. (1993). Chapter 18. Oxidative phosphorylation and photophosphorylation. *Principles of biochemistry.* Worth Publishers. N. Y. Second Edition. Pp 542, 543, 554, 559.

Leibovitz, B., M. L. Hu, and A. L. Tappel. (1990). Dietary supplements of vitamin E, beta-carotene, coenzyme Q_{10} and selenium protect tissue against lipid peroxidation in rat tissue slices. *J. Nutrition.* 120: 97-104.

Lemmi, C. A., P. C. Pelikan, B. Geesaman, E. Seamon, M. Koyle, and J. Raifer. (1990). Kinetics of cyclosporine A-induced inhibition of succinate-coenzyme Q dehydrogenase in rat renal cortical mitochondria. *Biochem. Med. Metab. Biol.* 43: 214-225.

Lensmeyer, G. L., D. A. Wiebe, and I. H. Carlson. (1988). Deposition of nine metabolites of cyclosporine in human tissues, bile, urine, and whole blood. In: *Deposition of CsA metabolites in human.* Pp. 614-622.

Lettéron, P., N. Brahimi-Bourouina, M.A. Robin, A. Moreau, G. Feldmann, and D. Pessayre. (1997). Gloucocorticoids inhibit mitochondrial matrix acyl-CoA dehydrogenases and fatty acid β-oxidation. *J. Appl. Physiol.* 272: G1141-1150.

Levine, S. M., A. Anzueto, J. I. Peters, T. Cronin, E. Y. Sako, S. G. Jenkinson, and C. L. Bryan. (1994). Medium term function results of single-lung transplantation for endstage obstructive lung disease. *Am. J. Respir. Crit. Care Med.* 150:398-402.

Levy, R. D., P. Ernst, S. M. Levine, H. Shennib, A. Anzueto, C. L. Bryan, J. H. Calhoon, J. K. Trinkle, S. G. Jenkinson, and W. J. Gibbons. (1993). Exercise performance after lung transplantation. *J. Heart Lung Transplant.* 12: 27-33.

Lim, P. O., R. J. MacFadyen, P. B. Clarkson, and T. M. MacDonald. (1996). Impaired exercise tolerance in hypertensive patients. *Ann. Intern. Med.* 124: 41-55.

Lizasoain, I., M. A. Moro, R. G. Knowles, V. Darley-Usmar, and S. Moncada. (1996). Nitric oxide and peroxnitrite exert distincts on mitochondrial respiration which are differentially blocked by glutathione or glucose. *Biochem. J.* 314: 877-880.

Lochner, A., N. van der Merwe, M. de Villiers, C. Steinmann, and J. C. Kotzyé. (1987). Mitochondrial Ca^{2+} fluxes and levels during ischaemia and reperfusion: possible mechanisms. *Biochem. Biophys. Acta* 927: 8-17.

Lo-Russo, A., A. C. Passaquin, P. Andre, M. Skutella, and U. T. Ruegg. (1996). Effect of cyclosporin A and analogues on cytosolic calcium and vasoconstriction: possible lack of relationship to immunosuppressive activity. *Br. J. Pharmacol.* 118: 885-892.

Lowenstein, J. M. (1972). Ammonia production in muscle and other tissues: the purine nucleotide cycle. *Physiol. Reviews* 52: 383-414.

Lowry, C. V., J. S. Kimmey, S. Felder, M. M. Y. Chi, K. I. Kaiser, P. N. Passonneau, K. A. Kirk, and O. H. Lowry. (1978). Enzyme patterns in single human muscle fibres. *J. Biol. Chem.* 253: 8269-8277.

Lowry, O. H., and J. V. Passoneau. (1972). Flexible system of enzymetic analysis. Academic press. New York and London. Pp. 199-200.

Lowry, O.H., N .J. Rosebrough, A. L. Farr, and R.J. Randall. (1951). Protein measurement with folin phenol reagent. *J Biol Chem.* 193: 265-275.

Luft, R. (1995). The development of mitochondrial medicine. Biochemca et Biophysica Acta 1271: 1-6.

Lundin, A. (1978). Determination of creatine kinase isoenzymes in human serum by an immunological method using purified firefly luciferase. *Methods Enzymol.* 57: 56-65.

Lundin, A., A. Rickardsson, and A. Thore. (1976). Continuous monitoring of ATP-converting reactions by purified firefly luciferase. *Analytical. Biolchem.* 75: 611-620.

Lüthi, J. M., H. Howald, H. Claassen, K. Rosler, P. Vock, and H. Hoppeler. (1986). Structural changes in skeletal muscle tissue with heavy rsistance exercise. *Int. J. Sports Med.* 7: 123-130.

MacDougall, J. D., A. L. Hicks, J. R. MacDounald, R. S. McKelvie, H. J. Green, and K. M. Smith. (1998). Muscle performance and enzymatic adaptation to sprint interval training. *J. Appl. Physiol.* 84: 2138-2142.

MacDougall, J. D., D. G. Sale, G. C. B. Elder, and J. R. Sutton. (1982). Muscle ultrastructural characteristics of elite powerlifter and bodybuilders. *Eur. J. Appl. Physiol.* 4 8:117-126.

MacDougall, J. D., D. G. Sale, J. R. Moroz, G. C. B. Elder, J. R. Sutton, and H. Howald. (1979). Mitochondrial volume density in human skeletal muscle following heavy resistance training. *Med. Sci. Sports* 11: 164-166.

MacDougall, J. D., G. C. B. Elder, D. G. Sale, J. R. Moroz and J. R. Sutton. (1980). Effects of strength training and immobilization on human muscle fibres. *Euro. J. Appl. Physiol. Occup. Physiol.* 43: 25-34.

MacDougall, J. D., G. R. Ward, D. G. Sale, and J. R. Sutton. (1977). Biochemical adaptation of human skeletal muscle to heavy resistance training and immobilization. *J. Appl. Physiol.* 43: 700-703.

MacDougall, J. D., M. J. Gibala, M. A. Tarnopolsky, J. R. MacDonald, S. A. Interisano, and K. E. Yarasheski. (1995). The time course for elevated muscle protein synthesis following heavy resistance exercise. *Can. J. Appl. Physiol.* 20: 480-486.

MacLean, D. A, L. L. Spriet, E. Hultman, and T. E. Graham. (1991). Plasma and muscle amino acid and ammonia responses during prolonged exercise in humans. *J. Appl. Physiol.* 70: 2095-2103.

Madden, B. P., M. E. Hodson, V. Tsang, R. S. Radley, A. Khaghani, and M. Y. Yacoub. (1992). Intermediate-term results of heart-lung transplantation for cystic fibrosis. *Lancet* 27, 339:1583-1590.

Madsen, K., P. Ertbjerg, M. S. Djurhuus, and P. K. Pedersen. (1996). Calcium content and respiratory control index of skeletal muscle mitochondria during exercise and recovery. *Am. J. Physiol.* 271: E1044-E1050.

Maganaris, C. N., and R. J. Maughan. (1998) Creatine supplementation enhances maximum voluntary isometric force and endurance capacity in resistance trained men. *Acta Physiol. Scand.*163: 279-287.

Magnusson, G., A. Gordon, L. Kaijser, C. Sylven, B. Isberg, J. Karpakka, and B. Saltin. (1996). High intensity knee extensor training, in patients with chronic heart failure. Major skeletal muscle improvement. *Eur. Heart J.* 17: 1048-1055.

Maiorana, A. J., T. G. Briffa, C. Goodman, and J. Hung. (1997). A controlled trial of circuit weight training on aerobic capacity and myocardial oxygen demond in men after coronary artery bybass surgery. *J. Cardiopulm. Rehabil.* 17: 234-247.

Makinen, M. W., and C. P. Lee. (1968). Biochemical studies of skeletal muscle mitochondria. I. Microanalysis of cytochrome content, oxidative and phosphorylative activities of mammalian skeletal muscle mitochondria. *Arch. Biochem. Biophys.* 126: 75-82.

Mal, H., C. Sleiman, G. Jebrak, O. Messian, F. Dubois, C. Darne, J. P. Duchatelle, J. L. Mollo, M. Fournier, M. Kitzis, B. Aadreassian, and R. Pariente. (1994). Functional results of single-lung transplantation for chronic obstructive lung disease. *Am. J. Respir. Crit. Care Med.* 149: 1476-1481.

Maltais, F., A. A. Simard, C. Simard, J. Jobin, P. Desgagns, and P. Leblanc, with R. Janvier. (1996). Oxidative capacity of the skeletal muscle and lactic acid kinetics during exercise in normal subjets and in patients with COPD. *Am. J. Respir Care. Med.* 153: 288-293.

Mancini D. M., E. Coyle, A Coggan,. J. N. Belts, S. Ferraro, Montain, and Wilson J. R. (1989). Contribution of intrinsic skeletal muscle changes to ^{31}P NMR skeletal muscle metabolic abnormalities in patients with chronoc heart failure. *Circulation* 80: 1338-1346.

Marcinik, E. J., J. Potts, G. Schlabach, S. Will, P. Dawson, and B. F. Hurley. (1991). Effects of strength training on lactate threshold and endurance performance. Med. Sci. Sports Exerc. 23: 739-743.

Massari, S., and G. F. Azzone. (1972). The equivalent pore radius of intact & damaged mitochondria and mechanism of active shrinkage. *Biochem. Biophys. Acta* 283: 23-29.

Massie, B. M., A. Simonini, P. Sahgal, L. Wells, and G. A. Dudley. (1996). Relation of systemic and local muscle exercise capacity to skeletal muscle characteristics in men with congestive heart failure. *J. Am. Coll. Cardiol.* 27: 140- 145.

Massie, B. M., M. Conway, B. Rajagopalan, R. Yonge, S. Frsotick, J. Ledingham, P. Sleight, and G. Radda. (1988). Skeletal muscle metabolism during exercise under ischemic condition in congestive `heart failure: evidence for abnormalities unrelated to blood flow. *Circulation* 78: 320-326.

Matsunaga, K., K. Nakatani, M. Ishibashi, J. Kobayashi, Y. Ohizumi, and B. Amphidinolide. (1999). A powerful activator of actomyosin ATPase enhances skeletal muscle contraction. *Biochim. Biophys. Acta* 14, 1427: 24-32.

Maurer, J. R., T. L. Winton, G. A. Patterson, and T. R. Williams. (1991). Single lung transplantation for pulmonary vascular disease. *Transplant Proc.* 23: 1211-1212.

Max, S. R. (1972). Disuse atrophy of skeletal muscle: loss of functional activity of mitochondria. *Biochem. Biophy. Res. Communi.* 46: 1394-1398.

Max, S. R., J. Garbus, and H. Wehman. (1972). Simple procedure for rapid isolation of functionally intact mitochondrial from human and rat skeletal muscle. *Anal. Biochem.* 46: 576-584.

McCall, G. E., W. C. Byrnes, A. Dickinson, P. M. Pattany, and S. J. Fleck. (1996). Musucle fibre hypertrophy, hyperplasia, and capillary density in college men after resistance training. *J. Appl. Physiol.* 81: 2004-2012.

McCartney, N. (1998). Role of resistance training in heart disease. *Med. Sci. Sports Exerc.* 30 (suppl): S396-S402.

McCartney, N., and R. S. McKelvie. (1996). The role of resistance training in patients with cardiac disease. *J. Cardiovasc. Risk.* 3:106-166.

McComas, A. J. (1994). Human neuromuscular adaptations that accompany changes in activity. *Med. Sci. Sports Exer.* 26: 1499-1509.

McCormack, J. G., and R. M. Denton. (1979). The effects of calcium ions and adenine nucleotides on the activity of pig heart 2-oxoglutarate dehydrogenase complex. *Biochem. J.* 180: 533-544.

McCutcheon, J., S. K. Byrd, and D. R. Hodgson. (1992). Ultrastructural changes in skeletal muscle after fatiguing exercise. *J. Appl. Physiol.* 73: 1111-1117.

McDonagh, M. J. N., and C. T. M. Davies. (1984). Adaptive response of mammalian skeletal muscle to exercise with high loads. *Eur. J. Appl. Physiol.* 52: 139-155.

McGarry, J. D., and D. W. Foster. (1980). Regulation of hepatic fatty acid oxidation and ketone body production. *Annu. Rev. Biochem.* 49: 395-420.

McMillin, J. B., M. C. Madden. (1989). The role of calcium in the control of respiration by muscle mitochondria. *Med. Sci. Sports Exerc.* 21: 406-410.

MedbØ, J. I., and I. Tabata. (1989). Relative importentnce of aerobic and anaerobic energy release during short-lasting exhausting bicycle exercise. *J. Appl. Physiol.* 67: 1881-1886.

Mercier, J. G., J. F.Hokanson, and G. A. Brooks. (1995). Effects of cyclosporine A on skeletal muscle mitochondrial respiration and endurance time in rats. *Am. J. Respir. Crit. Care Med.* 151: 1532-1536.

Metzger, J. M., and R. H. Fitts. (1987). Role of intracellular pH on muscle fatigue *J. Appl. physiol.* 62: 1392-1397.

Minotti, J., R. I. Christoph, and B. M. Massie. (1992). Skeletal muscle function, morphology, and metabolism in patients with congestive heart failure. *Chest* 101: 333-339.

Miquel, J., A. C. Economos, J. Fleming, and J. E. Johnson, Jr. (1980). Mitochondrial role in cell aging. *Exp. Gerontol.* 15: 575-591.

Miro, O, A. Barrientos, J. R. Alonso, J. Casademont, D. Jarreta, A. Urbano-Marquez and F. Cardellach. (1999). Effects of general anaesthetic procedures on mitochondrial function of human skeletal muscle. *Eur. J. Clin. Pharmacol* 55: 35-41.

Mitchell, P. (1961). Coupling of phosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism. *Nature* 191: 144-148.

Mitchell, P. (1979). Keilin's respiratory chain comcept and its chemiosmotic consequences. *Science* 206: 1148-1159.

Mitchell, P., and J. Moyle. (1967). Resiratory-driven proton translocation in rat liver mitochondria. *Biochem. J.* 105: 1147-1162.

Miyoshi, S., E. P. Trulock, H. J. Schaefers, C. M. Hsieh, G. A. Patterson, and J. D. Cooper. (1990). Cardiopulmonary exercise testing after singe and double lung transplantation. *Chest* 97: 1130-1136.

Mizuno, M. (1991). Human respiratory muscles: fibre morphology and capillary supply. *Eur. Respir. J.* 4: 587-601.

Mole, P. A., L. B. Oscai, and J. O. Holloszy. (1971). Adaptation of muscle to exercise. Increase in levels of palmity CoA synthesas, carnitine palmityltransferase, and palmityl CoA dehydrogenase and in the capacity to oxidize fatty acids. *J. Clin. Invest.* 50: 2323-2330.

Morrison, J. F. J., T. W. Higenbottam, T. J. Hathaway, C. Clelland, J. P. Scott, and J. Wallwork. (1992). Diurnal variation in FEV_1 after heart-lung transplantation. *Eur. Respir. J.* 5: 834-840.

Morrison, R. J., H. D. Short, G. P. Noon, and A. E. Frost. (1993). Hypertension after lung transplantation. *J. Heart Lung Transplant* 12: 928-931.

Morton J. M., M. J. McKenna, M. F. Carey, S. F. Fraser, J. A. Parkin, G. I. Snell, M. Rabinov, J. A. Smith, D. S. Esmore, and T. J. Williams. (1997). Alterations in skeletal

muscle fibre types in chronic obstructive airways disease (COPD) – a consequence of altered muscle activity? (1997). 10th Int. Biochemistry of Exercise Conf., The University of Sydney, July 15-19, P11, Proc.

Moss, B. M., P. E. Refsnes, A. Abildgaard, K. Nicolaysen and J. Jensen. (1997). Effects of maximal strength training with different loads on dynamic strength, cross-sectional area, load-power and load velocity relationships. *Eur. J. Appl. Physiol.* 75: 193-199.

Myers, B. A., T. R. Brown, B. L. Krilowicz, and M. J. Kushmerick. (1986). Phosphagen and intracellular pH changes during contraction of creatine-depleted rat muscle. *Am. J. Physiol.* 250: C264-C274.

Myers, B. D., L. Newton, C. Boshkos, J. A. Macoviak, W. H. Frist, G. C. Derby, M. G. Perlroth, and R. K. Sibley. (1988). Chronic injury of human renal microvessels with low-dose cyclosporine therapy. *Transplantation* 46: 694-703.

Narici, M. V., H. Hoppeler, B. Kayser, L. Landoni, H. Claassen, C. Gavardi, M. Conti, and P. Cerretelli. (1996). Human quadriceps cross-sectional area, torque and neural activation during 6 months strength training. *Acta Physiol. Scand.* 157: 175-186.

National Heart, Lung and Blood Institute (NHLBI) Workshop Summary. (1990). Respiratory muscle fatigue. *Am. Rev. Respir. Dis.* 142: 474-480.

Nava, S., G. Gayan-ramirez, H. Rollier, A. Bisschop, R. Dom, V. D. Bock, and M. Decrame. (1996). Effects of acute steroid administration on ventilatory and periperal muscle in rats. *Am. J. Respir. Crit. Care Med.* 153: 1888-1896.

Näveri, H. K., H. Leinonen, K. Kiilavuori, and M. Härkönen. (1997). Skeletal muscle lactate accumulation and creatine phosphate depletion during heavy exercise in congestive heart failure. *Eur. Heart J.* 18: 1937-1945.

Newsholme, E. A. (1980). Use of enzyme activity measurements in studies on the biochemistry of exercise. *Int. J. Sports. Med.* 1: 100-102.

Newsholme, E. A., and A. R. Leech. (1983). Chapter 4. The oxidation of Acetyl-coenzyme A. In: *Biochemistry for the Medical Sciences*. J. Wiley and sons. Great Britain. Pp. 101.

Newsholme, E. A., and C. Start. (1973). Regulation in metabolism. J. Wiley and sons (eds.) London, UK. Pp. 112.

Nicholls D. G. (1978). The regulation of extra-mitochondrial free calcium ions concentration by rat liver mitochondria. *Biochem. J.* 176: 463-474.

Nielsen, O. B., and T. Clausen. (1997). Regulation of Na^+-K^+ pump activity in contracting rat muscle. *J. Physiol.* 15 503: 571-581.

Nimmo, M. A., and D. H. Snow. (1982). Time course of ultrastructural changes in skeletal muscle after two types of exercise. *J. Appl. Physiol.* 52: 910-913.

Noppen, M., B. Velkeriers, R. Dierckx, M. Bruyland, and I. Vanhaclat. (1987). Cyclosporine and myopathy. *Am. Intern. Med.* 107: 945-946.

Norman, B. (1994). IMP accumulation in energy deficient human skeletal muscle. Stockholm. Pp. 2-38.

Norman, B., P. Hedén, and E. Jansson. (1991). Small accumulation of inosine monophosphate (IMP) despite high lactate levels in latissimus dorsi during transplantation. *Clin. Physiol.* 11: 375-384.

Novelli, G. P., C. Adembri, E. Gandini, S. Z. Oriandini, L. Papucci, L. Formigli, L. I. Manneschi, A. Quattrone, C. Pratesi, and S. Capaccioli. (1997). Vitamin E protects human skeletal muscle from damage during surgical ischaemia-reperfusion. *Am. J. Surg.* 172: 206-209.

Nygaard, E. (1981). Skeletal muscle fiber characteristics in young women. *Acta Physiol. Scand.* 122: 299-304.

Odedra, B. R., P. C. Bates, and D. J. Millward. (1983). Time course of the effect of catabolic doses of corticosterone on protein turnover in rat skeletal muscle and liver. *Biochem. J.* 214: 617-627.

Orens, J. B., F. S. Becker, J. P. Lynch-3rd, P. J. Christensen, G. M. Deeb, and F. J. Martinez. (1995). Cardiopulmonary exercise testing following allogeneic lung transplantation for different underlying disease states. *Chest* 107: 144-153.

Orlander, J., K. H. Kiessling, L. Larsson, J. Karlsson, and A. Aniansson. (1978). Skeletal muscle metabolism and ultrastructure in relation to agae in sedentary men. *Acta Physiol. Scand.* 104: 249-261.

Orrenius, S., D. J. McConkey, G. Bellomo, and P. Nicotera. (1989). Role of Ca^{2+} in toxic cell killing. *Trends Pharmacol. Sci.* 10: 281-285.

Osumi, T., and T. Hashimoto. (1980). Purification and properties of mitochondrial and peroxisomal 3-hydroxyacylt-CoA dehydrogenase from rat liver. *Arch. Biochem. Biophys.* 203: 372-383.

Oyer, P. E., E. B. Stinson, S. W. Jamieson, S. A. Hunt, M. Perlronth, M. E. Billinhgam, and N. E. Shumway. (1983). Cyclosporin in cardiac transplantation: a $2^1/_2$ year follow-up. *Transplant Proc.* 15: 2546-2552.

Packer, L. (1997). Oxidants, antioxidant nutrients and the athlete. *J. Spo. Sci.* 15: 353-363.

Palade, G. E. (1953). An electron microscope study of mitochondrial structure. *J. Histochem. Cytochem.* 1:188-211.

Palou, A., C. Pico, M. L. Bonet, and P. Oliver. (1998). Uncoupling protein, thermogenin. *Int. J. Biochem. Cell. Biol.* 30: 7-11.

Panegyres, P. K., M. Squeir, and K. R. Mills. (1993). Acute myopathy associated with large parenteral dose of corticosteroid in myasthenia gravis. *J. Neurol. Neurosury. Psychiatry.* 56:702-704.

Parsons, D. F., G. R. Williams, W. Thompson, D. Wilson, and B. Chance. (1967). Improvents in the procedure for purification of outer and inner membrane. Comparison of the out membrane with smooth endoplasmic reticulum, in *Mitochondrial Structure and Compartmentation* Quagliariello, E., S. Papa, E. C. Slater, and J. M. Tager. (eds.) Adriatica Press, Bari, Pp. 29-73.

Pasque, M. K., E. P. Trulock, J. D, Cooper, A. N. Triantafillou, C. B. Huddleston, M. Rosenbloom, S. Sundaresan, J. L. Cox, and G. A. Patterson. (1995). Single lung transplantation for pulmonary hypertension. Single institution experience in 34 patients. *Circulation.* 92: 2252-2258.

Patterson, G. A., J. D. Cooper, B. Goldman, R. D. Weisel, F. G. Pearson, P. F. Waters, T. R. Todd, H. Scully, M. Goldberg, and R. J. Ginsberg. (1988). Technique of successful clinical double lung transplantation. *Ann. Thorac. Surg.* 45: 626-632.

Patterson, G. M., S. Wilson, J. L. Whang, J. Harvey, K. Agacki, H. Patel, and J. Theodore. (1996). Physiologic definitions of obliterative bronchiolitis in heart-lung and double lung transplantation: a comparision of the forced expiratory flow between 25% and 75% of the forced vital capacity and forced expiratory volume in one second. *J. Heart Lung Transplant.* 15: 175-181.

Payen, J. F., B. Wuyam, P. Levy, H. Reutenauer, P. Steiglitz, B. Paramelle, and J. F. Le Bas. (1993). Muscular metabolism during oxygen supplementation in patients with chronic hypoxemia. *Am. Rev. Respir. Dis.* 147: 592-598.

Pei, Y., C. Chan, D. Cattran, C. Cardella, J. Zaltzman, M. Lopez, J. Tong, R. Schachter, and J. Maurer. (1995). Sustained vasoconstriction associated with daily cyclosporine dose in heart and lung transplant recipients: potential pathophysiologic role of endothelin. *J. Lab. Clin. Med.* 125: 113-119

Peter, J. B., D. A. Verhaag, and M. Wordford. (1970). Studies of steroid myopathy: Examination of the possible effect of triamcinolone on mitochondria and darcotubular vesicies of rat skeletal muscle. *Biochem. Pharmac.* 19: 1627-1636.

Petronilli, V., C. Cola, and P. Bernardi. (1993). Modulation of the cyclosporine A-sensitive permeability transition pore. II. The minimal requirents for pore induction underscore A key role for transmembrane electrical, potential, matrix pH, and matrix Ca^{2+} *J. Biol. Chem.* 268: 1011-1016.

Pollock, M. L., G. A. Gasser, J. D. Butcher, J. P. Després, R. K. Dishman, B. A. Frankin, and C. E. Garber. (1998). The recommended quantity and quality of exercise for developing and maintaing cardiorespiratory and muscle fitness, and fleibility in healthy adults. *Med. Sci. Sports Exerc.* 30: 975-991.

Pouw E. M., A. M. W. J. Schols, G. J. V. D.Vusse, and E. F. M. Wouters. (1998). Elevated inosine monophosphate levels in resting muscle of patients with stable chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 157: 453-457.

Qian, T., A. L. Nieminen, B. Herman, And J. J. Lemasters. (1997). Mitochondrial permeability transition in pH-dependent reperfusion injury to rat hepatocytes. Am. J. Physiol. 273: C1783-C1792.

Racker, E., D. D. Tyler, R. W. Estabrook, T. E. Conover, D. F. Parsons, and B. Chance. (1965). Correlation between electron transport activity, ATPase and morphology of submitochondrial particles, in *Oxidases and Related Redox Systems.* T.E. King, H. S. Mason and M. Morrison. (eds.). J. Wiley and Sons. New York. Pp. 1077-1094.

Read, G., G. Crabtree, and G. H. Smith. (1977). The activities of 2-oxoglutarate dehydrogenase and pyruvate dehydrogenase in hearts and mammary glands from ruminants and non-ruminants. *Biochem. J.* 164: 349-355.

Reichenspurner, H., R. E. Girgis, R. C. Robbins, J. V. Conte, R. V. Nair, V. Valentine, G. J. Berry, R. E. Morris, J. Theodore, and B. A. Reitz. (1995). Obliterative bronchiolitis after lung and heart-lung transplantation. *Ann. Thorac. Surg.* 60: 1845-1853.

Reitz, B. A., J. L. Wallwork, S. A. Hunt, J. L. Pennock, M. E. Billingham, P. E. Oyer, E. B. Stinson, and N. E. Shumway. (1982). Heart lung transplantation: successful therapy for patients with pulmonary vascular disease. *N. Eng. J. Med.* 306: 557-564.

Ren, J. M., and E. Hultman. (1989). Regulation of glycogenolysis in human skeletal muscle. *J. Appl. Physiol.* 67: 2243-2248.

Richter C., M. Theus, and J. Schlegel. (1990). Cyclosporine A inhibits mitochondrial pyridine nucleotide hydrolysis and calcium release. *Biochem. Pharmacol.* 40: 779-782.

Richter, C., V. Gogvadze, R. Laffranchi, R. Schlapbach, M. Schweizer, M. Suter, P. Walter, and M. Yaffee. (1995). Oxidants in mitochondria: from physiology to diseases. *Biochimica et Biophysica Acta* 1271: 67-74.

Rieder, M. J., D. M. O'Drobinak, P. J. Tonellato, and A. S. Greene. (1996). Mathematical analysis of type-I and type-IIb muscle fibre force generation in renal hypertension. *Ann. Biomed. Eng.* 24: 489-499.

Rifenberick, D. H., and S. R. Max. (1974). Substrate utilization by disused rat skeletal muscles. *Am. J. Physiol.* 226: 295-297.

Ritchie, M., A. D., Waggoner, V. G. Davila-Roman, B. Barzilai, E. P. Trulock, and P. R. Eisenberg. (1993). Echocardiographic characterization of the improvement in right ventricular function in patients with severe pulmonary hypertension after single-lung transplantation. *J. Am. Coll. Cardiol.* 22: 1170-1174.

Robergs, R. A., and S. Roberts. (1997). Glossary. In *Exercise physiology Exercise perforamce and clinical applications.* Robergs, R. A., and S. Roberts (Eds.). Mosby-year book Inc. Von hoffimann press Inc. St. louis, Missourri, USA. Pp 803-811.

Rolfe, D. F. S., A. J. Hulbert, and M. D. Brand. (1994). Characteristics of mitochondrial proton leak and control of oxidative phosphrylation in major oxygen consuming tissues of the rat. *Biochem. Biophys. Acta* 1188: 405-416.

Rolfe, D. F. S., and M. D. Brand. (1996). Contribution of mitochondrial proton leak to skeletal muscle respiration and to standard metabolisc rate. *Am. J. Physiol.* 271: C1380-C1389.

Rolfe, D. F., and G. C. Brown. (1997). Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol. Rev.* 77: 731-758.

Rolfe, D. F., J. M. Newman, J. A. Buckingham, M. G. Clark, and M. D. Brand. (1999). Contribution of mitochondrial proton leak to respiration rate in working skeletal muscle and liver and to SMR. Am. J. Physiol. 276: C692-C699.

Ronquist, G., B. Soussi, G. Frithz, T. Scherstén, and A. Waldenstrm. (1995). Distured energy balance in skeletal muscle of patients with untreated primary hypertension. *J. Int. Med.* 238: 167-174.

Rooyackers, O. E., D. B. Adey, P. A. Ades, and K. S. Nair. (1996). Effect of age on in vivo rates of mitochondrial protein synthesis in human skeletal muscle. *Proc. Natl. Acad. Sci.* 93: 15364-15369.

Ross, D. J., P. F. Waters, Z. Mohsenifar, M. J. Belman, R. M. Kass, and S. K. Koerner. (1993). Hemodynamic responses to exercise after lung transplantation. *Chest* 103: 46-53.

Rube, N., and N. H. Secher. (1991). Effect of training on central factors in fatigue following two and one-leg static exercise in man. *Acta Physiol. Scand.* 141: 87-95.

Rudas, L., P. W. Pflugfelder, F. N. McKenzie, A. H. Menkis, R. J. Novick, and W. J. Kostuk. (1992). Normalization of upright exercise hemodynamics and improved exercise capacity one year after orthotopic cardiac transplantation. *Am. J. Cardiol.* 69: 1336-1339.

Sahlin, K. (1992). Metabolic factors in fatigue. *Sports Med.* 13: 99-107.

Sahlin, K., A. Alverstrand, R. Brandt, and E. Hultman. (1978). Intracellular pH and bicarbonate concentration in human muscle during recovery from exercise. *J. Appl. Physiol.* 45: 474-480.

Sahlin, K., and A. Katz, and S. Broberg. (1990). Tricarboxylic acid cycle intermediates in human muscle during prolohged exercise. *Am. J. Physiol.* 259: C834-C841.

Sahlin, K., and A. Katz. (1989). Hypoxaemia increases the accumulation of inosine monophosphate (IMP) in human skeletal muscle during submaximal exercise. *Acta Physiol. Scand.* 136: 199-203.

Sahlin, K., and S. Broberg. (1990). Adenine nucleotide depletion in human muscle during exercise: causality and significance of AMP deamination. *Int. J. Sports Med.* 11 (Suppl 2): S62-S67.

Sahlin, K., K. Söderlund, M. Tonkonogi, and K. Hirakoba. (1997). Phosphocreatine content in single fibers of human muscle after sustained submaximal exercise. *Am. J. Physiol.* 273: C172-C178.

Sahlin, K., M. Tonkonogi, and K. Söderlund. (1998). Energy supply and muscle fatigue in humans. *Acta Physiol. Scand.* 162: 261-266.

Sahlin, K., S. Broberg, and J. M. Ren. (1989). Formation of inosine monophosphate (IMP) in human skeletal muscle during incremental dynamics exercise. *Acta Physiol. Scand.* 136: 193-198.

Salaman, J. R. (1984). Cyclosporine in renal transplantation: a guide to management. *Lancet* 2, 8397: 269-271.

Salducci, M. D., A. M. Chauvet-Monges, J. P. TillemntY, E. Albengres, Berland, B. Testa, P. Carrupt, and A. Crevat. (1996). Trimetazidine reverses calcium accumulation and impairment of phosphorylation induced by cyclosporine A in isolated rat live mitochondria. *J. Pham. Exp. Therap.* 277: 417-422.

Sale, D. G., A. J. McComas, J. D. MacDougall, and A. R. M. Upton. (1982). Neuromuscular adaptation in human thenar muscles following strength training and immobilization. *J. Appl. Physiol.* 53: 419-424.

Saltin, B. (1973). Metabolic fundamentals in exercise. *Med. Sci. Sports.* 5: 137-146.

Saltin, B., J. Henriksson, E. Nygaard, and P. Anderson. (1977). Fibre types and metabolic potentials of skeletal muscle in sedentary man and endurance runners. *Annal. N. Y. Acad. Sci.* 301: 3-29.

Samuta, T., G. Becker, R. Pohorecki, R. Bahar, and D. Landers. (1993). Cyclosporine, isoflurane, and oxygenation effects on energy balance in isolated hepatocytes. *Transplant. Proc.* 25: 1847-1849.

Sant'Ana Pereira, J. A. A., A. J. Sargeant, A. C. H. Rademaker, A. de Haan, and W. van Mechelen. (1996). Myosin heavy chain isoform expression and high energy phosphate content in human muscle fibre at rest and post-exercise. *J. Physiol.* 496: 583-588.

Sapangenburg, E. E., C. W. Ward, and J. H. Williams. (1998). Effects of lactate on force production by mouse EDL muscle: implications for the development of fatigue. *Can. J. Physiol. Pharmacol.* 76: 642-648.

Sarris, G. E., J. A. Smith, N. E. Shumway, E. B. Stinson, P. E. Robbins, M. E. Billingham, J. Theodore, K. A. Moore, and B. A. Reitz. (1994). Long-term results of combined heart-lung transplantation: the Standord experience. *J. Heart Lung Transplant.* 13: 940-949.

Sato, Y, T. Asoh, Y. Honda, Y. Fujimatsu, I Higuchi, and K. Oizumi. (1997). Morphologic and histochemical evaluation of muscle in patients with chronic pulmonary emphysema manifesting generalized emaciation. *Eur. Neurol.* 37: 116-121.

Savage, M. K., D. P. Jones, and D. J. Reed. (1991). Calcium- and phosphate-dependent release and loading of glutathione by liver mitochondria. *Arch. Biochem. Biophys.* 290: 51-56.

Savin, W. H., W. L. Haskell, J. S. Schroeder, and E. B. Stinson. (1980). Cardiopulmonary response to graded, symptom-limited exercise. *Circulation.* 62: 55-60.

Schantz, P. G., and M. Källman. (1989). NADH shuttle enzymes and cytochrome *b5* reductase in human skeletal muscle: effect of strength training. *J. Appl. Physiol.* 67: 123-127.

Schantz, P., B. Sjöberg, A. M. Widebeck, and B. Ekblom. (1997). Skeletal muscle of trained and untrained paraplegics and tetraolegics. *Acta Physiol. Scand.* 161: 31-39.

Schmidt, E. (1974). Glutamate Dehydrogenase. In: *Methods of enzymatic analysis.* H. U. Bergmeyer, (ed.) 2nd ed. Academic, New York: Pp. 650-656.

Schulz. H. (1991). Beta-oxidation of fatty acids. *Biochem. Biophys. Acta.* 1081: 109-121.

Sciurba, F. C., G. R. Owens, M. H. Sanders, B. P. Griffith, L. R. H. Hardesty, I. L. Paradis and J. P. Costantino. (1988). Evidence of an alteraed pattern of breathing during exercise in recipients of heart-lung transolants. *New Eng. J. Med.* 319: 1186-1192.

Scott, J. P., B. A. Otulana, P. A. Mullins, D. J. Aravoj, T. Higenbottam, and J. Wallwork. (1992). Late pulmonary haemodynamic changes in heart-lung transplantation. *Eur. Heart J.* 13: 503-507.

Sen, C. K. (1995). Oxidants and antioxidants in exercise. *J. Appl. Physiol.* 79: 675-686.

Sen, C. K., T. Rankinen, S. Väisänen, and R. Rauramaa. (1994). Oxidative stress after human exercise: effect of *N*-acetylcysteine supplementation. *J. Appl. Physiol.* 76: 2570-2577.

Shapiro, B. L. (1989). Evidence for a mitochondrial lesion in cystic fibrosis. *Life Sci.* 44: 1327-1334.

Shee, C. D. (1990). Risk factors for hydrocortisone myopathy in acute severe asthma. *Resp. Med.* 84: 229-233.

Shepherd, P. and P. B. Garland. (1969). The kinetic properties of citrate synthase from rat mitochondria. *Methods Enzymol.* 13: 11-16.

Sherratt, H. S. A. (1991). Mitochondria: Structure and function. *Rev. Neurol.* 147: 417-430.

Sherratt, H. S. A., M. A. Johnson, and D. M. Turnbull. (1988). Methods for study of narmal and abnormal skeletal muscle mitochondria. *Methods Biochem. Analys.* 33: 243-335.

Shigenaga, M. K., C. J. Gimeno, and B. N. Ames. (1989). Urinary 8-hydroxy-2'-deoxyguanosine as a biological marker of in vivo oxidative DNA damage. *Proc. Natl. Acad. Sci.* 86: 9697-9701.

Siegel, H., B. Pyffel, P. Petric, F. Fumagalli, I Brown, P. Donatsch and m. Mihatsch. (1983). Cyclosporine, the renin-angiotensin-aldosterone system, and renal adverse reactions. *Tansplantat Proc.* 15; 2719-2725.

Simon, N., R. Zini, C. Morin, F. Brée and J. Tillement. (1997). Prednisolone and azathioprine worsen the cyclosporine a-induced oxidative phosphorylation decrease of kidney mitochondria. *Life Sci.* 61: 659-666.

Singer, S. J., and G. L. Nicolson. (1972). The fluid mosaic model of structure of cell membranes. *Science* 175: 720-731.

Sipilä, S., and H. Suominen. (1995). Effects of strength and endurance training on thigh and leg muscle mass and composition in elderly women. *J. Appl. Physiol.* 78: 334-340.

Siri, W. E. (1956). Gross composition of the body. In: *Advances in Biological and Medical Physics*, IV, J.H. Lawrence and C.A. Tobias (Eds.). New York: Academic Press.

Skelton, D. A., A. Young, C. A. Greig, and K. E. Malbut. (1995). Effects of resistance training on strength, power, and selected functional abilities of women aged 75 and older. *J. Am. Geriatr. Soc.* 43: 1081-1087.

Smith, P. F., R. S. Eydelloth, S. J. Grossman, J. Stubbs, M. S. Schwartz, J. I. Germershausen, K. P. Vyas, P. H. Kari, and J. S. Macdonald. (1991). HMG-CoA reductase inhibitor-induced myopathy in rat: cyclosporine A interaction and mechanism studies. *J. Pharm. Exp. Therap.* 257:1225-1235.

Snell, G. I., C. Ward, J. W. Wilson, B. Orsida, T. J. Williams and E. H. Walters. (1997). Immunopathological changes in the airways of stable lung transplant recipients. *Thorax* 52: 322-328.

Söderlund, K., and E. Hultman. (1991). ATP and phosphocreatine changes in single human muscle fibers following intense electrical stimulation. *Am. J. Physiol.* 261: C71-C76.

Sohal, R. S., and A. Dubey. (1994). Mitochondrial oxidative damage, hydrogen peroxide release, and aging. *Free Radic. Biol. Med.* 16: 621-626.

Soussi, B., J. P. Idström, T. Scherstén, and A. C. Bylund-Fellenius. (1990). Cytochrome C oxidase and cardiolipin alterations in response to skeletal muscle ischaemia and reperfusion. *Acta Physiol. Scand.* 138: 107-114.

Spangenburg, E. E., C. W. Ward, and J. H. Williams. (1998). Effects of lactate on force production by mouse EDL muscle: implications for the development of fatigue. *Can. J. Physiol. Pharmacol.* 76: 642-648.

Spencer, M. K., Z. Yan, and A. Katz. (1991). Carbohydrate supplementation attenuates IMP accumulation in human muscle during prolonged exercise. *Am. J. Physiol.* 261: C71-C76.

Spiro, S. G. (1977). Exercise testing in clinical medicine. *Br. J. Dis. Chest* 71: 145-172.

Spray, T. L. (1993). Projections for pediatric heart-lung and lung transplantation. *J. Heart Lung Transplant.* 12: S337-5343.

Srere, P. A. (1969). Citrate synthase. In: *Methods in Enzymology.* S. P. Colowick, and N. O. Kaplan. (eds.). Academic Press, New York. Pp. 3-5.

Stadtman, E. R. (1992). Protein oxidation and aging. *Science* 257: 1220-1224.

Stadtman, E. R., and C. N. Oliver. (1991). Metal-catalyzed oxidation of proteins physiological consequences. *J. Biol. Chem.* 266: 20005-20008.

Starkey, D. B., M. L. Pollock, Y. Ishida, M. A. Welsch, W. F. Brechue, J. E. Graves, and M. S. Feigenbaum. (1996). Effect of resistance training volume on strength and muscle thickness. *Med. Sci. Sports Exerc.* 28: 1311-1320.

Starnes, V. A., M. L. Barr, and R. G. Cohen. (1994). Lobar transplantation. indications, technique, and outcome. *J. Thorac. Cardivasc. Surg.* 108:403-410.

Staron, R. S., and D. Pette. (1990). The multiplicity of myosin light and heavy chain combinations in muscle fibres. In: *The dynamic state of muscle fibres.* D. Pette (ed.). Walter de Gruyter. Berlin. Pp. 315-328.

Staron, R. S., D. L. Karapondo, W. J. Kraemer, A. C. Fry, S. E. Gordon, Falkel-JE, F. C. Hagerman, and R. S. Hikida. (1994). Skeletal muscle adaptations during early phase of heavy-resistance training in men and women. *J. Appl. Physiol.* 76: 1247-1255.

Staron, R. S., E. S. Malichy, M. J. Leonard, J. E. Falkel, F. C. Hagerman, and G. A. Dudley. (1990). Muscle hypertrophy and fast fiber type conversions in heavy resistance-trained women. *Eur. J. Physiol. Occup. Physiol.* 60: 71-79.

Staron, R. S., M. J. Leonard, D. L. Karapondo, E. S. Malicky, J. E. Falkel, F. C. Hagerman, and R. S. Hikida. (1991). Strength and skeletal muscle adaptations in heavy-resistance-trained women after deteraining and retraining. *J. Appl. Physiol.* 70: 631-640.

Staron, R. S., R. S. Hikida, F. C. Hagerman, G. A. Dudley, and T. F. Murray. (1984). Human skeletal muscle fiber type adaptability to various workloads. *J. Histochem. Cytochem.* 32: 146-152.

Stathis, C. G., M. A. Febbraio, M. F. Carey, and R. J. Snow. (1994). Influence of sprint training on human skeletal muscle purine nucleotide metabolism. *J. Appl. Physiol.* 76: 1802-1809.

Steenbergen, C., E. Murphy, J. A. Watts, and R. E, London. (1990). Correlation between cytosolic free calcium, contracture, ATP, and irreversible ischaemic injury in perfused rat heart. *Circ. Res.* 66: 135-146.

Stratton, J. R., G. J. Kemp, R. C. Daly, M. Yacoub, and B. Rajagopalan. (1994). Effects of cardiac transplantation on bioenergetic abnormalities of skeletal muscle in congestive heart failure. *Circulation* 89: 1624-1631.

Sullivan, M. J., H. J. Green, and F. R. Cobb. (1990). Skeletal muscle biochemistry and histology in ambulatory patients with long-term heart failure. *Circulation* 81: 518-527.

Sumida, S., K. Tanaka, H. Kitao, and F. Nakadomo. (1989). Exercise-induced lipid peroxidation and leakage of enzymes before and after vitamin E supplementation. *Interna. J. Biolchem.* 21: 835-838.

Suter, E., H Hoppeler, H. Claassen, R. Billeter, U. Aebi, F. Horber, P. Jaeger and B. Marti. (1995). Ultrastructural modification of human skeletal muscle tissue with 6-month moderate-intensity exercise training. *Int. J. Sports Med.* 16: 160-166.

Svedenhag, J., J. Henriksson, and A. Juhlin-Dannfelt. (1984). β-adrrenergic blockade and training in human subjects: effects on muscle metabolic capacity. *Am. J. Physiol.* 247: E305-E311.

Svendsen, U., H. Arendrup, M. Norgaard, P. Olsen, J. Thiis, S. Mortensen, and G. Pettersson. (1995). Double lung transplantation with bronchial artery revascularization using mammary artery. *Transplant Proc.* 27: 3485.

Takeda, Y., I. Miyamori, P. Wu, T. Yoneda, K. Furukawa, and R. Takeda. (1995). Effects of an endothelin receptor antagonist in rats with cyclosporine-induced hypertension. *Hypertension* 26: 932-936.

Tanaka, T., K. Hosaka, M. Hoshimura, and S. Numa. (1979). Purification and properties of long-chain acyl-coenzyme-A synthetase from rat liver. *Eur. J. Biochem.* 98: 165-172.

Tanaka, T., S. Hakoda, and N. Takeyama. (1998). Reoxygen-induced mitochondrial damage is caused by the Ca2+-dependent mitochondrial inner membrane permeability transition. *Free Radic. Biol. Med.* 25: 26-32.

Terjung, R. L., K. M. Baldwin, P. A. Mole, G. H. Klinkerfuss, and J. O. Holloszy. (1972). Effect of running to exhaustion on skeletal muscle mitochondria: a biochemical study. *Am. J. Physiol.* 223:549-554.

Tesch, P. A. (1988). Skeletal muscle adaptations consequent to long-term heavy resistance exercise. *Med. Sci. Sports Exerc.* 20 (Suppl): S132-S134.

Tesch, P. A., A. Thorsson, and B. Essén-Gustavsson. (1989). Enzyme activities of FT and ST muscle fibers in heavy rsistance trained athletes. *J. Appl. Physiol.* 67: 83-87.

Tesch, P. A., A. Thorsson, and E. B. Colliander. (1990). Effects of eccentric and concentric resistance training on skeletal muscle substrates, enzyme activities and capillary supply. *Acta Physiol. Scand.* 140: 575-80

Tesch, P. A., E. B. Colliander, and P. Kaiser. (1986). Muscle metabolism during intense, heavy rsistance exercise. *Eur. J. Appl. Physiol.* 55: 362-366.

Tesch, P. A., P. V. Komi, and K. Häkkinen. (1987). Enzymatic adaptations consequent to long-term strength training. *Int. J. Sports Med.* 8: 66-69.

Textor, S.C., V. J. Canzanello, S. J. Taler, D. J. Wilson, L. L. Schwartz, J. E. Augustine, J. M. Raymer, J. C. Romero, R. H. Wiesner, and R. A. Krom. (1994). Cyclosporine-induced hypertension after transpalntation. *Mayo Clin. Proc.* 69: 1182-1193.

Theodore, J., A. J. Morris, C. M. Burke, A. R. Glanville, A. Vankessel, J. C. Baldwin, E. B. Stinson, N. E. Shumway, and E. D. Robin. (1992). Cardiopulmonary function at maximum tolerable constant work rate exercise following human heart-lung transplantation. *Chest* 92: 433-439.

Thompson L. V. (1994). Effects of age and training on skeletal muscle physiology and performance. *Phys. Ther.* 74: 71-81.

Thorstensson, A., B. Hulten, W. von-Dobeln, and J. Karlsson. (1976b). Effect of strength training on enzyme activities and fibre characteristics in human skeletal muscle. *Acta Physiol. Scand.* 96: 392-398

Thorstensson, A., Grimby, G., and J. Karlsson. (1976a). Force-velocity relations and fiber compositions in human knee extensor muscles. *J. Appl. Physiol.* 40, 12-16.

Tonkonogi, M., and K. Sahlin. (1997). Rate of oxidative physphorylation in isolated mitochondria from human skeletal muscle: effect of training status. *Acta. Physol. Scand.* 161: 345-353.

Tonkonogi, M., B. Harris, and K. Sahlin. (1998). Mitochondrial oxidative function in human saponin-skinned muscle fibres: effects of prolonged exercise. *J. Physiol.* 510, 279-286.

Tonkonogi, M., B. Walsh, T. Tiivel, V. Saks, and K. Sahlin. (1999). Mitochondrial function in human skeletal muscle is not impaired by high intensity exercise. *Pflugers Arch.* 437: 562-568.

Toronto Lung Transplant Group. (1986). Unilateral lung transplantation for pulmonary fibrosis. *N. Eng. J. Med.* 314: 1140-1145.

Town, G.P., and D.A. Essig. (1993). Cytochrome oxidase in muscle of endurance-trained rat: subunit mRNA contents and heme synthesis. *J. Appl. Physiol.* 74: 193-196.

Trounce, I., E. Byrne, and S. Marzuki (1989). Decline in skeletal muscle mitochondrial respiratory chain function: possible factor in ageing. *Lancet* 25(8639): 637-639.

Trumpower, B. L. (1990). The protonmotive Q cycle: energy transduction by coupling of proton translocation to electron transfer by the cytochrome bc_1 complex. *J. Biol. Chem.* 265: 11409-11412.

Tsuchiya, M., M. Takahashi, M. Tomoda, W. Ueda and M. Hirakawa. (1990). Halothane impairs the bioenergetic functions of isolated rat liver mitochondria. *Toxicol. appl. Pharmacol.* 104: 466-475.

Tuena De Gomes-Puyou, M., M. Gavhilanes, A. Gomes-Puyou, and A. Lars Esster. (1980). Control of activity states of heart mitochondrial ATPase: Role of the proton-motive force and Ca^{++}. *Biochem. Biophys. Acta* 582: 396-405.

Turner, D. L., H. Hoppeler, H. Classen, P. Vock, B. Kayser, F. Schena, and G. Ferretti. (1997). Effects of ehdurance training on oxidative capacity and structural composition of human and leg muscles. *Acta Physiol. Scand.* 161: 459-464.

Tyler, D. D. (1995). Chapter 4 The mitochondrion. In: *Principless of Medical Biology, vol. 3. Cellular Organelles and the Extracellular Matrix.* Bittar, E. E., and N. Bittar. (Eds.). JAI Press INC. England. Pp. 59-132.

Van Balkom, R. H. H., W. Z. Zhan, Y. S. Prakash, P. N. Richard Dekhuijzen, and G. C. Sieck. (1997). Corticosteroid effects on isotonic contractile properties of rat diaphragm muscle. *J. Appl. Physiol.* 83: 1062-1067.

Vanden Hoek, T. L., Shao, Zuohui, Li, Changqing, Schumacker, P. T. and L. B. Becker. (1997). Mitochondrial electron transport can become a significant source of oxidative injury in cardiomyocytes. *J. Mol. Cell. Cardiol.* 29: 1441-2450.

Vandenberghe, K., M. Goris, P. Van hecke, M, Van Leemputte, L. Vangerven, and P. Hespel. (1997). Long-term creatine intake is beneficial to muscle performance during resistance training. *J. Appl. Physiol.* 83: 2055-2063.

Veitch, K., A. Hombroeckx, D. Caucheteux, H. Poulrur, and L. Hue. (1992). Global ischaemia induces a biphasic response of the mitochondrial respiratory chain. Anoxic pre-perfusion protects against ischaemic damage. *Biochem. J.* 181: 709-715.

Verrill, D. E., and P. M. Ribisl. (1996). Resistive exercise training in cardiac rehabilitation. An update. *Sports Med.* 21: 347-83

Vidal-Puig, A., G. Solanes, D. Grujic, J. S. Flier, and B. B. Lowell. (1997). UCP3: an uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue. *Biochem. Biophys. Res. Commun.* 235: 79-82

Wagenmakers, A. J., E. J. Beckers, F. Brouns, H. Kuipers, P. B. Soeters, G. J. Van der vusse, and W. H. M. Saris. (1991). Carbohydrate supplementation, glycogen depletion, and amino acid metabolism during exercise. *Am. J. Physiol.* 260: E883-E890.

Wagner, O., E. Schreier, F. Heitz, and G. Maurer. (1987). Tissue distribution, disposition, and metabilism of cyclosporine in rats. *Drug metabolism & Disposition* 15: 377-383.

Walker, J. E., R. Lutter, A. Dupuis, and M. J. Runswick. (1991). Identification of the subunits of F_1Fo-ATPase from bovine heart mitochondria. *Biochemistry* 30: 5369-5378

Walker, P. D., C. Das, and S. V. Shah. (1986). Cyclosporine A induce lipid peroxidation in renal cortical mitochondria. *Kidney Int.* 29: 311-319.

Walker, P. M., F. Brunotte, I. Rouhier-Marcer, Y. Cottin, J. M. Castillas P. Gras, and J. P. Didier. (1998). Nuclear magnetic resonace evidence of different muscular adaptation after resistance training. *Arch. Phys. Med. Rehabil.* 79: 1391-1398.

Walker, R. J., V. A. Lazzaro, G. G. Duggin, J. S. Horvath, and D. J. Tiller. (1990). Evidence that alteration in renal metabolism and lipid peroxidation may contribute to cyclosporine nephrotoxicity. *Transplantation* 50: 487-492.

Wang, N., R. S. Hikida, R. S. Staron and A. Simoneau. (1993). Muscle fiber types of women after resistance training-quantitative ultrastructure and enzyme activity. *Pflugers Arch.* 424: 494-502.

Wasserman, K., B. J. Whipp, S. N. Koyal, and W. L. Beaver. (1973). Anaerobic threshold and respiratory gas exchange during exercise. *J. Appl. Physiol.* 35: 236-243.

Weiss, H., T. Friedrich, G. Hofhaus, and D. Preis. (1991). The respiratory chain NADH dehydrogenase (Complex I) of mitochondria. *Eur. J. Biochem.* 197: 563-576.

Welsh D. G., and M. I. Lindinger. (1997). Metabolite accumulation increases adenine nucleotide degradation and decreases glycogenolysis in ischaemic rat skeletal muscle. *Acta Physiol. Scand.* 161: 203-210.

Westerblad, H., J. D. Bruton, and J. Lannergren. (1996). The effect of intracellular pH on contractile function of intact single fibres of mouse muscle declines with increasing trmperature. *J. Physiol.* 500: 193-204.

Westerblad, H., J. A. Lee, J. Lännergren, and D. G. Allen. (1991). Cellular mechanisms of fatigue in skeletal muscle. *Am. J. Physiol.* 261: C195-C209.

Whittom, F., J. Jobin, P. Simard, P. Leblanc, C. Simard, S. Bernard, R. Belleau and F. Maltais. (1998). Histochemical and morphological characteristics of the vastus lateralis muscle in patients with chronic obstructive pulmonary disease. *Med. Sci. Sports Exerc.* 30: 1467-1474.

Wibom, R., A. Lundin, and E. Hultman. (1990). A sensitive method for measuring ATP-formation in rat muscle mitochondria. *Scand. J. Clin. Lab. Invest.* 50: 143-152.

Wibom, R., and E. Hultman. (1990). ATP production rate in mitochondria isolated from microsamples of human muscle. *Am. J. Physiol.* 259:E204-E209.

Wibom, R., E. Hultman, M. Johansson, K. Matherei, D. Constantin-teodosiu, and P.G. Schantz. (1992). Adaptation of mitochondrial ATP production in human skeletal muscle to endurance training and detraining. *J. Apple. Physiol.* 73: 2004-2010.

Wicks, K. L., and D. A. Hood. (1991). Mitochondrial adaptations in denervated muscle: relationship to muscle performance. *Am. J. Physiol.* 260: C841-C850.

Wilcox, P. G., J. M. Hards, K. Bockhold, B. Bressler, and R. L. Pardy. (1989). Pathologic changes and contractile properties of the diaphragm in corticosteroid myopathy in hamsters: Comparison to peripheral muscle. *Am. J. Respir. Cell Mol. Biol.* 1: 191-199.

Williams, T. J., and G. I. Snell. (1997). Early and long-term functional outcomes in unilateral, bilateral, and living-related transplant recipients. *Clin. Chest. Med.* 18: 245-257.

Williams, T. J., G. A. Patterson, P. A. McClean, N. Zamel, and J. R. Maurer. (1992a). Maximal exercise testing in single and double lung transplant recipients. *Am. Rev Respir. Dis.* 145:101-105.

Williams, T. J., M. Howard, J. Roget, and D. S. Esmore. (1992b). Exercise capacity after combined heart-lung transplantation. *Transplant Proc.* 24:2018.

Williams, T. J., R. E. O'Hehir, D. Czarny, M. Horne, and G. Bowes. (1988). Acute myopathy in severe acute asthma treated with intravenously administered corticosteroids. *Am. Rev. Respir. Dis.* 137: 460-463.

Williams, T. J., R. F. Grossman, and j. R. Maurer. (1990). Long-term functional follow-up of lung transplant recipients. *Clin. Chest Med.* 11: 347-358.

Willis, W. T., and M. R. Jackman. (1994). Mitochondrial function during heavy exercise. *Med. Sci. Sports Exerc.* 26: 1347-1353.

Wilson, D. E. (1994). Factors affecting the rate and energetics of mitochondrial oxidative phosphorylation. *Med. Sci. Sports Exerc.* 26: 37-43.

Wilson, J. R., J. L. Martin, D. Schwartz, and N. Ferraro. (1984). Exercise intolerance in patients with chronic heart failure: role of impaired nutritive flow in skeletal muscle. *Circulation* 69: 1079-1087.

Wilson, P. D., and L. M. Franks. (1975). The effect of age on mitochondrial ultrastracture and enzymes. *Adv. Exp. Med. Biol.* 53: 171-183.

Winter, J. B., A. S. Gouw, M. Groen, C. Wildevuur, and J. Prop. (1994). Respiratory viral infections aggravate airway damage caused by chronic rejection in rat lung allografts. *Transplantation* 57: 418-422.

Wolf, A., C. F. Treendelenburg, C. Diez-Fernandez, P. Prieto, S. Houy, W. E. Trommer, and A. Cordier. (1997). Cyclosporine A-induced oxidative stress in rat hepatocytes. *J. Pharm. Exper. Therap.* 280:1328-1334.

Wolf, A., N. Clemann, W. Frieauff, B. Ryffel, and A. Cordier. (1994). Role of reactive oxygen formation in the cyclosporine-A-mediated impairment of renal functions. *Transplant Proc.* 26: 2902-2907.

Woodson, R. D., R. E. Wills, and C. Lenfant. (1978). Effect of acute and established anaemia on O_2 transport at rest, submaximal and maximal work. *J Appl Physiol.* 44: 36-43.

Wynants, J., and H. Van Bell. (1985). Single-run high performance liquid chromatography of nucleotides, nucleosides, and major purine bases and its application to different tissure extracts. *Anal. Biochem.* 144: 258-266.

Yajid, F., J. G. Mercier, B. M. Mercier, H. Dubouchaud, and C. Prefaut. (1998). Effects of 4 wk of hindlimb suspension on skeletal muscle mitochondrial respiration in rats. *J. Appl. Physiol.* 84: 479-485.

Yeaman, S. J. (1989). The 2-oxo acid dehydrogenase complexes: recent advances. *Biochem. J.* 257: 625-632.

Zamzami N., P. Marchetti, M. Castedo, D. Decaudin, A. Macho, T. Hirsch, S. A. Susin, P. X. Petit, B. Mignotte, and G. Kroemer. (1995). Sequential reduction of mitochondrial transmembrane potential and generation of reactive oxygen species in early programmed cell death. *J. Exp. Med.* 182: 367-377.

Zenatti, M., B. Aupetti, A. Ghazzi, E. Shechter, A. Michel, V. Nataf, P. Aymard, and J. C. Legrand. (1988). Is the inhibition of oxidative phosphorylation chains in kidney mitochondria responsible for cyclosporine nephrotoxicity. *Transplant. Proc.* (XX suppl). 3: 700-704.

Zhang, Y., O. Marcillat, C. Giulivi, L. Ernster, and K. J. A. Davies. (1990). The oxidative inactivation of mitochondrial ETC components and ATPase. *J. BioChem.* 265: 16330-16336.

APPENDIX

APPENDIX I. INFORMATION CONSENT

VICTORIA UNIVERSITY OF TECHNOLOGY STANDARD CONSENT FORM FOR SUBJECTS INVOLVED IN EXPERIMENTS

CERTIFICATION BY SUBJECT

I,.....of.....
certify that I have the legal ability to give valid consent and that I am voluntarily giving my consent to participate in the experiment entitled :

"Effects of Resistance Training on Human Skeletal Muscle Ionic Regulation and Mitochondrial Function."

being conducted at Victoria University of Technology by :
Associate Professor Michael F. Carey, Dr Michael J. McKenna
I certify that the objectives of the experiment, together with any risks to me associated with the procedures listed hereunder to be carried out in the experiment, have been fully explained to me by
.....and that I freely consent to participation involving the use on me of these procedures.

Procedures:

Anthropometric measurements, including underwater weighing
Lung function measurements
Maximal incremental exercise test on a cycle ergometer
Venous catheterisation and blood sampling during incremental exercise test
Biodex muscle function tests
Muscle biopsies at rest and following fatiguing knee extensor exercise on Cybex.
Venous catheterisation and blood sampling during Cybex fatigue test
I certify that I have had the opportunity to have my questions answered and that I understand that I can withdraw from the experiment at any time and that this withdrawal will not jeopardise me in any way.
I have been informed that the confidentiality of the information I provide will be safeguarded.

Signed : .....Witness other than the experimenter Darte : / /
(day/month/year)

VICTORIA UNIVERSITY OF TECHNOLOGY

Subject Information Sheet

"Effects of Resistance Training on Human Skeletal Muscle Ionic Regulation and Mitochondrial Function."

CHIEF INVESTIGATORS:
Dr Michael J. McKenna, Assoc. Prof. Michael F. Carey,
Department of Physical Education & Recreation, Department of Chemistry & Biology, Victoria University of Technology, Footscray, VIC.

Aim of the study:

This study will compare muscle size, biochemistry, maximal strength and the development of fatigue during repeated contractions, between strength-trained an untrained individuals.

Subject participation:

As a volunteer to participate as a control subject, you are free to withdraw from the study at any time, without any adverse effects, reactions or discrimination.

Exercise Testing and Lung Function Testing Procedures:

You will be required to attend the Exercise Physiology Laboratory (Room L329, Building L), at the Footscray Campus of Victoria University of Technology on <u>two</u> occasions. On the first visit to the laboratory you will be required to perform a maximal incremental exercise test on a cycle ergometer to evaluate your maximal aerobic exercise characteristics. Blood samples will be taken from a hand vein at rest, during and following exercise to indicate your metabolic response to exercise. During this visit we will also measure the capacity of your lungs and the rate at which you can breath air in and out, by getting you to blow air in/out of a special instrument. We will also determine the size of your leg muscles by measuring the girth, length and volume of your leg and your lean .

Muscle Function Testing
Your maximal thigh muscle strength will be determined using a Biodex isokinetic dynamometer located in the C.R.E.S.S. biomechanics laboratory at 300 Flinders Street, City Campus. The test will require you to perform several maximal contractions at different speeds of contraction. This will be followed by a test to fatigue your thigh muscles, involving 50 repeated contractions of your leg muscles.

Blood Samples:
Blood samples (each 5 ml) will be drawn from a catheter inserted into a vein on the back of your hand. A catheter is a thin, flexible, sterile piece of tubing which sits inside your vein. With the use of a small tap, blood samples can be drawn from the catheter at specific times. A total of around fifty (50) ml of blood will be taken during the test. (Normally 400 ml is taken when you donate at the Blood Bank). Blood samples will be analysed for haemoglobin, haematocrit, plasma pH, gases, ions, lactate and other metabolites.
The catheters used for drawing blood are routinely used for clinical purposes and exercise testing; however, slight bruising may occur at the site of catheter insertion. On very rare occasions, blood clots may form. Emergency equipment will be present at all tests. To avoid any risk of blood borne diseases all catheters, syringes and disposable items will be sterile, not previously used and discarded immediately following use.

Muscle Biopsies and Muscle Fatigue Testing:
On the second visit to the Human Performance Laboratory, Footscray Campus, a muscle biopsy will be taken from your thigh muscle whilst you are lying on a couch. You will then be asked to perform the muscle fatigue test involving 50 repeated contractions of your thigh muscle, on the Cybex dynamometer. A muscle biopsy will be taken immediately after exercise finishes. Muscle biopsies are routinely carried out in our laboratory, with no adverse effects. The three possible complications with a muscle biopsy are pain, infection and internal bleeding. To prevent you from suffering pain, a local anaesthetic (needle) will be given in the skin overlying your thigh muscle. To prevent infection, only sterilised instruments will be used. To prevent excessive bleeding, pressure will be manually applied after biopsies have been taken and maintained through use of a pressure bandage. In addition, no major nerves or blood vessels are located in the vastus lateralis muscle that we biopsy.

By signing the informed consent form you are indicating that the tests and procedures have been explained to you and are understood by you. Also, it is accepted by the investigators and by yourself that you are participating voluntarily in the study and that you are free to withdraw from the investigation at any time. Thank you for your co-operation.

CARDIOVASCULAR RISK FACTOR QUESTIONNAIRE
In order to be eligible to participate in the experiment investigating:
"Effect of Resistance Training on Human Skeletal Muscle
Ionic Regulation and Mitochondrial Function"
you are required to complete the following questionnaire which is designed to assess the risk of you having a cardiovascular event occurring during an exhaustive exercise bout.
Name: _____ Date: _____ Age: _____ years
Weight: _____ kg Height: _____ cms
Give a brief description of your average activity pattern in the past 2 months:
_____

Circle the appropriate response to the following questions.
1. Are you overweight? Yes No Don't know
2. Do you smoke? Yes No Social
3. Does your family have a history of premature cardiovascular problems (eg. heart attack, stroke)? Yes No Don't Know
4. Are you an asthmatic Yes No Don't Know
5. Are you a diabetic? Yes No Don't Know
6. Do you have a high blood cholesterol level? Yes No Don't Know
7. Do you have high blood pressure? Yes No Don't Know
8. Are you on any medication? Yes No
   If so, what is the medication? _____
9. Do you think you have any medical complaint or any other reason which you know of which you think may prevent you from participating in strenuous exercise? No
   Yes, please elaborate _____

I, _____, believe that the answers to these questions are true and correct.

Signed: _____ Date: _____

MUSCLE BIOPSY QUESTIONNAIRE
NAME: _____

ADDRESS: _____

DATE: _____ AGE: _____ years

1. Have you or your family suffered from any tendency to bleed excessively ? (eg. haemophilia) or bruise very easily ?

   Yes No Don't Know

   If yes, please elaborate..._____

2. Are you allergic to local anaesthetic?

   Yes No Don't Know

   If yes, please elaborate..._____

3. Do you have any skin allergies?

   Yes No Don't Know

   If yes, please elaborate..._____

4. Have you any allergies that should be made known?

   Yes No Don't Know

   If yes, please elaborate... _____

5. Are you currently on any medication?

   Yes No Don't Know

   If yes, what is the medication? _____

6. Do you have any other medical problem that should be made known?

   Yes No

   If yes, please elaborate... _____

To the best of my knowledge, the above questionnaire has been completely accurately and truthfully.

Signature: _____ Date: _____

MUSCLE BIOPSY PROCEDURE

The muscle biopsy is a relatively painless procedure that is used to obtain small samples of skeletal muscle tissue for metabolic analysis. A small incision is made in the skin overlying the muscle, under local anaesthetic. The biopsy needle is then inserted into the muscle and a small piece of tissue removed from the muscle. During this part of the procedure you may feel some pressure and a tendency for the muscle to cramp, however, this only persists for a few seconds. Following the biopsy the incision will be closed using a steri-strip and a pressure bandage will be applied which should be maintained for 24-48 hours. Steri-strip closure should be maintained for a few days. You should not exercise for 24-48 hours after biopsies and you should avoid heavy knocks. It is common for subjects to experience some mild soreness in the muscle over the next 2-3 days, however this passes and does not restrict movement. In some rare cases mild haematomas have been reported, but these symptoms disappear within a week. The whole procedure will be preformed under sterile conditions by a qualified medical practitioner. Although the possibility of infection and significant bruising is quite small, if by chance it does eventuate, inform us immediately and then consult your doctor.

CATHETERISATION

At specific intervals throughout the exercise test a small blood sample will be taken via a catheter placed into a hand vein. The catheter consists of a needle and teflon tubing. The tubing is fed over the top of the needle (which has punctured the vein) and into the vein. The needle is then withdrawn, leaving only the teflon tubing in your vein for the remainder of the experiment. A tap (stopcock) is placed into the tubing so the flow of blood along the tubing can be altered at will. This procedure allows the taking of multiple blood samples without the need for multiple venepunctures (puncturing of the vein). Each time a blood sample is taken, a small volume of sterile heparinised saline will be injected to clear the catheter and keep it patent. Catheterisation of subjects is slightly discomforting and can lead to the possibility of bruising and infection. The use of sterile, disposable catheters, syringes, swabs etc. will markedly reduce the possibility of infection caused by the catheterisation procedure. The use of qualified and experienced staff will reduce the likelihood of bruising as this is primarily caused by poor venepuncture techniques. Although the possibility of infection and significant bruising is quite small, if by chance it does eventuate, inform us immediately and then consult your doctor.

VICTORIA UNIVERSITY OF TECHNOLOGY

STANDARD CONSENT FORM FOR SUBJECTS INVOLVED IN EXPERIMENTS

CERTIFICATION BY SUBJECT

I,.....of..... certify that I have the legal ability to give valid consent and that I am voluntarily giving my consent to participate in the experiment entitled :
"Skeletal muscle analysis post thoracic transplantation: control subjects" being conducted at Victoria University of Technology by :
Dr Michael J. McKenna, Assoc. Prof. Michael F. Carey, Dr Trevor Williams, Dr Michael Hall

I certify that the objectives of the experiment, together with any risks to me associated with the procedures listed hereunder to be carried out in the experiment, have been fully explain.....and that I freely consent to participation involving the use on me of these procedures.

Procedures

I certify that I have had the opportunity to have my questions answered and that I understand that I can withdraw from the experiment at any time and that this withdrawal will not jeopardise me in any way.

I have been informed that the confidentiality of the information I provide will be safeguarded.

Signed : .....Witness other than the experimenter : Date : .....

VICTORIA UNIVERSITY OF TECHNOLOGY

SUBJECT INFORMATION SHEET

"Skeletal muscle analysis post thoracic transplantation: control subjects"

INVESTIGATORS:
Dr Michael J. McKenna, Assoc. Prof. Michael F. Carey,
Department of Physical Education & Recreation, Department of Chemistry & Biology, Victoria University of Technology, Footscray, Vic.

Dr Trevor Williams, Dr Michael Hall
Department of Respiratory Medicine, The Alfred Hospital, Prahran, Vic.

Aim of the study:

This study will compare your muscle composition and metabolism, as well as maximal exercise performance, with that of patients that have undergone heart, lung or heart and lung transplants. The study is designed to determine why these transplant patients have a reduced exercise performance.

Subject participation:

As a volunteer to participate as a control subject, you are free to withdraw from the study at any time, without any adverse effects, reactions or discrimination.

Exercise Testing Lung Function Testing Procedures:

On two occasions, separated by a four week time period, you will be required to attend the Exercise Physiology Laboratory (Room L329, Building L), at the Footscray Campus of Victoria University of Technology. On the first visit to the laboratory you will be required to perform a maximal incremental exercise test on a cycle ergometer to evaluate your maximal aerobic exercise characteristics. Blood samples will be taken from a hand vein at rest, during and following exercise to indicate your metabolic response to exercise.

We will measure the capacity of your lungs and the rate at which you can breath air in and out, by getting you to blow air in/out of a special instrument. This carries no additional risk.

Blood samples:

Blood samples (each 5ml) will be drawn from a catheter inserted into a distal forearm vein. A catheter is a thin, flexible, sterile piece of tubing which sits inside your vein. With the use of a small tap, blood samples can be drawn from the catheter at specific times. A total of around fifty (50) ml of blood will be taken during the test. Blood samples will be analysed for haemoglobin, haematocrit, plasma pH, gases, ions, lactate and other metabolites.

The catheters used for drawing blood are routinely used for clinical purposes and exercise testing; however, slight bruising may occur at the site of catheter insertion. On very rare occasions, blood clots may form. Emergency equipment will be present at all tests. To avoid any risk of blood borne diseases all catheters, syringes and disposable items will be sterile, not previously used and discarded immediately following use.

Muscle Biopsies:

On the second visit to the laboratory, a muscle biopsy will be taken from your thigh muscle whilst you are lying on a couch. Muscle biopsies are routinely carried out in our laboratory, with no adverse effects. The three possible complications with a muscle biopsy are pain, infection and internal bleeding. To prevent you from suffering pain, a local anaesthetic (needle) will be given in the skin overlying your thigh muscle. To prevent infection, only sterilised instruments will be used. To prevent excessive bleeding, pressure will be manually applied after biopsies have been taken and maintained through use of a pressure bandage. In addition, no major nerves or blood vessels are located in the vastus lateralis muscle that we biopsy.

By signing the informed consent form you are indicating that the tests and procedures have been explained to you and are understood by you. Also, it is accepted by the investigators and by yourself that you are participating voluntarily in the study and that you are free to withdraw from the investigation at any time. Thank you for your co-operation.

APPENDIX II. EQUATIONS FOR PREDICTED VALUES, CHARACTERISTICS OF SUBJECTS AND EXERCISE PERFORMANCE

Characteristics of subjects, results of spirometry and anthropometric measurements and exercise performance. The methods of spirometric and anthropometric measurements have been described in relevance chapter. Equations for predicted values of VC and FEV_1 (Crapo et al 1981), TLCO (Crapo & Morris 1981), $\dot{V}O_2$ peak (Jones et al 1985) and HRpeak (Spiro 1977) are listed as below:

$\dot{V}C$ equation for males $\dot{V}C(L) = 0.06 \times \text{high (cm)} - 0.0214 \times \text{age} - 4.65.$

$\dot{V}C$ equation for female $\dot{V}C(L) = 0.0491 \times \text{high (cm)} - 0.0216 \times \text{age} - 3.59.$

FEV_1 equation for males $FEV_1 = 0.0414 \times \text{high (cm)} - 0.0244 \times \text{age} - 2.19.$

FEV_1 equation for Females $FEV_1 = 0.0342 \times \text{high (cm)} - 0.0255 \times \text{age} - 1.578.$

TLco peak equation for males $TLCO = 0.416 \times \text{high (cm)} - 0.219 \times \text{age} - 26.34.$

TLcopeak equation for females $TLCO = 0.256 \times \text{high (cm)} - 0.1444 \times \text{age} - 8.36.$

$\dot{V}O_2$peak equation for males $\dot{V}O_2\text{peak} = 3.45\times\text{high (cm)} - 0.028\times\text{age} + 0.022\times\text{weight (kg)} - 3.76L$ min^{-1}.

$\dot{V}O_2$peak equation for females: $\dot{V}O_2\text{peak} = 2.49\times\text{high (cm)} - 0.018\times\text{age} + 0.010\times\text{weight (kg)} - 2.26L$ min^{-1}.

HRpeak equation for male and females $HRpeak = 210 - 0.65 \times \text{age (year)}.$

Body fat percentage (% BF) was calculated according to sum of 8 skinfold thicknesses using the following regression equations for males (Jackson & Pollock 1978) and females (Jackson et al.1980).

Regression equation for males $BD = 1.112000 - 0.0004399\,(X_1) + 5.5\ 10^{-7}(X_1)^2 - 0.00028826(X_2).$

BD = body density, X_1 = sum of tricep, bicep, subscapular, mid-axilla, suprailiac, abdominal, anterior thigh and medial calf (Sum of 8), X_2 = age (years).

Regression equation for females $BD = 1.096095 - 0.0006952\,(X_1) + 1.1*10^{-6}(X_1)^2 - 0.0000714(X_2).$

BD = body density, X_1 = sum of tricep, bicep, subscapular, mid-axilla, suprailiac, abdominal, anterior thigh and medial calf. X_2 = age (years)

The % body fat was calculated for both males and females using the following equation % BF = [(4.95/BD) – 4.50] × 100 (Siri 1956).

Estimation of body fat was also determined by underwater densitometry based on equation 6.3. (Brozek et al. 1963). Thigh volume was determined by water displacement.

Equation - underwater densitometry method.

$$\text{Body density} = \frac{BM}{\dfrac{BM - UWM}{DH_2O} - RLV}$$

BM = body mass (kg), UWM = underwater mass, DH_2O = density of water (at submersion temperature) and RLV = residual lung volume estimated from Forced Vital Capacity. Estimation of percentage body fat was calculated from the body density determination (Brozek, 1963).

Characteristics of subjects and spirometry in LTx and MC

Subjects	Sex	Age (year)	Height (cm)	Weight (kg)	VC (L)	Pred (L)	%	FEV1 (L)	Pred. (L)	%	TLCO ml min^{-1}	Pred. mmHg	%
LTx1	M	53	176	86.5	4.51	5	90	2.08	3.6	58	16.1	29	55.5
LTx2	F	49	167	56.4	2.22	3.61	61	1.36	2.75	49	10.2	24.9	41.0
LTx3	F	43	161	64.4	2.46	3.47	71	2.36	2.75	86	17.4	24.5	71.0
LTx4	F	34	172	62.6	4.23	4.17	101	3.48	3.35	104	20	28	71.4
LTx5	F	26	159	73.6	3.82	3.79	101	3.24	3.32	98	18.2	26.8	67.9
LTx6	M	29	178	59.4	3.6	5.63	64	3.4	4.54	75	25.2	34.4	73.3
LTx7	M	25	175	61.7	3.38	5.58	61	2.56	4.61	56	18.7	34.5	54.2
MC1	M	53	165	65.74	4.22	4.12	103	3.79	3.35	113	-	-	-
MC2	F	46	167	64.03	4.69	3.62	130	3.55	2.96	120	-	-	-
MC3	M	26	182	84.6	5.2	5.71	91	4	4.71	85	-	-	-
MC4	F	43	171	65.46	4.22	3.88	109	3.27	3.17	103	-	-	-
MC5	F	35	167	62.88	5.52	3.85	143	4.37	3.24	135	-	-	-
MC6	F	28	160	56.17	4.61	3.66	126	3.77	3.18	119	-	-	-
MC7	M	27	190	87.2	5	6.23	80	4.35	5.06	86	-	-	-

FEV_1 = forced expiratory volume in one second. VC = vital capacity. Pred. = predicted value. % = measured value / predicted value %. TLCO = diffusion capacity for carbon monoxide.

Medications used in addition to immunosuppressants in LTx

LTx1	cardizem	Bactrim					
LTx2		Gangclovir Pentamidine	Losec	Provera	Magmin	Quinine	Prothiaden
LTx3		Bactrim Minocycline	Zentac	Deseril		Calcium	Triphasil
LTx4	Renitec	Bactrim			Magmin	Calcium	Zestril
LTx5	Renitec	Bactrim	Zantac	lasix	Slow K	caltrate	Rocalcitrol
LTx6		itraconazole	Zantac	Magmin			Cotazyme
LTx7		Bactrim	Losec	Magmin			Cotazyme

Characteristics of subjects, results of spirometry and anthropometric measurements in RT and UT

Subjects	Sex	Age (year)	Height (cm)	Weight (kg)	VC (L)	FEV_1 (L)	Thigh Volume(L)	M+B Volume (L)	Water Displace (L)	Thigh M. C.S.A (cm^2)	BF % (UWW)	BF % (Sumof 8)
RT1	M	20	171	82	5.2	4.0	5.57	5.05	6.34	229.33	21.28	14.4
RT2	M	37	174	88	3.7	3.0	5.78	5.05	5.54	255.81	14.24	11.2
RT3	M	25	176	77	5.5	4.5	5.32	4.64	5.05	214.00	21.16	10.1
RT4	M	20	183	79	4.8	4.0	5.41	4.81	5.54	204.88	8.23	8.4
RT5	M	18	173	82	6.1	5.1	4.87	4.12	5.10	226.27	9.46	10.8
RT6	M	39	173	81	5.2	4.3	6.19	5.50	6.34	242.01	16.82	11.6
RT7	M	20	173	99	4.7	4.2	6.4	5.27	6.86	268.3	20.89	17
RT8	M	26	184	83	7.7	6.3	5.57	4.99	6.34	227.18	14.24	12.7
UT1	M	24	186	83	5.0	4.7	5.51	4.48	5.94	210.99	16.24	13.9
UT2	M	20	172	65	5.1	4.4	4.17	3.51	4.26	169.23	8.40	8.3
UT3	M	26	182	85	5.2	4.0	7.22	5.81	6.93	234.45	20.69	16.1
UT4	M	27	185	83	5.8	4.1	6.09	4.96	5.94	204.48	20.61	14.0
UT5	M	27	191	87	5.0	4.4	5.47	4.31	5.45	186.24	18.71	15.1
UT6	M	27	189	81	7.1	5.3	5.46	4.75	5.94	198.70	21.53	15.4
UT7	M	26	181	81	4.4	3.6	5.83	4.61	5.84	193.71	18.70	21.2
UT8	M	34	181	79	5.2	4.3	6.53	5.61	6.78	216.40	15.05	12.1

BF% (Sum of 8) = body fat calculation based on skinfolds. BF%(U.W.W).= body fat calculation based on under water weight. M.= muscle C.S.A.= muscle cross-sectional area.

Anthropometric measurements — skinfolds (mm) in RT and UT

Subjects	Tricep	Bicep	Subscap	Mid axilla	Supralliac	Abdominal	Anterior thigh	Medial calf	Sum of 8
RT1	6.30	7.03	16.37	12.50	20.40	22.20	7.60	11.10	103.50
RT2	3.80	2.80	10,90	11.15	12.05	14.45	11.20	3.90	70.25
RT3	4.30	11.50	9.70	8.20	11.50	13.70	10.70	6.70	76.30
RT4	5.50	3.30	8.33	7.10	9.25	9.00	8.90	4.95	56.33
RT5	7.85	4.10	8.60	6.85	11.95	13.45	13.30	7.75	73.85
RT6	8.05	2.85	9.60	7.30	16.30	12.65	9.85	5.30	71.90
RT7	10.4	3.1	16.60	15.0	24.2	21.5	18 0	14 3	123.0
RT8	4.85	3.20	12.60	8.40	20.00	19.80	8.40	6.10	83.35
UT1	10.93	5.65	9.90	7.90	12.10	23.13	16.30	8.20	94.12
UT2	7.00	3.37	6.87	5.33	7.10	7.13	11.73	7.03	55.57
UT3	11.50	5.70	12.90	10.87	23.30	22.10	17.80	12.50	116.67
UT4	12.10	3.60	8.67	6.97	10.15	22.50	15.87	8.00	87.85
UT5	10.35	5.80	11.45	7.20	15.20	22.40	18.20	18.65	109.25
UT6	6.20	5.10	11.10	9.40	22.25	31.50	10.93	7.10	103.58
UT7	15.50	8.60	25.27	17.60	27.30	32.30	17.50	9.45	153.52
UT8	7.95	3.10	11.90	9.05	9.75	15.20	11.70	5.80	74.45

Sum of 8 = summary of 8 location skinfolds.

APPENDIX III. FORMULA FOR MAPR CALCULATION

Mitochondrial ATP production rate was calculated using following formula which were obtained from Rolf Wibom's.

(1). **Mitochondrial ATP production rate in cuvette (MAPRc).**

MAPRc (nmol min^{-1} L^{-1}) = Δ **light** (arbitrary unit-AU) ÷ Δ **time** (minutes-min) × **[ATP]** (nmol ATP L^{-1}) /Δ **St** (AU).

Δ **light and** Δ **time** = Increase in light emission (Δ light) during a certain time (Δ time) as a result of `mitochondrial ATP production. **[ATP]** = Increase in ATP concentration in cuvette as a result of the addition of ATP standard. Δ **St** = Increase in light emission as a result of the addition of ATP standard.

(2). **Mitochondrial ATP production rate in suspension (MAPRs).**

MAPRs (mmol min^{-1} L^{-1}) = **MAPRc** × **FV** (final volume in cuvette, μl) × **D** (dilution factor for mitochondrial suspension before addition to cuvette) ÷ **SV** (Volume of diluted mitochondrial suspension added to cuvette, μl) × 10^{-6}.

(3) **Mitochondrial ATP production rate expressed per kg muscle (MAPRm)**

MAPRm (mmol min^{-1} kg^{-1}) = **MAPRs** ÷ **F** (see formula 6)

(4) **Mitochondrial ATP production rate expressed per gram mitochondrial protein (MAPRp).**

MAPRp (mmol min^{-1} g^{-1} protein) = **MAPRs** ÷ **prot** (protein concentration in mitochondrial suspension—g protein L^{-1})

(5). **Intact mitochondrial GDH activity in suspension (GDHim)**

GDHim (mmol min^{-1} L^{-1}) = **GDHma** (mmol min^{-1} L^{-1}) – **GDHmb** (mmol min^{-1} L^{-1}).

GDHma = GDH activity in mitochondrial suspension after disruption of mitochondrial membranes with Triton X-100). GDHmb = GDH activity in mitochondrial suspension before disruption of mitochondrial membranes with Triton X-100).

(6). **Ratio between intramitochondrial GDH activity in suspension and in muscle (F).**

F (kg L^{-1}) = **GDHim** (mmol min^{-1} L^{-1}) ÷ **GDHt** (total GDH activity in muscle, mmol min^{-1} kg^{-1}).

(7). **Yield of intact mitochondrial in the preparation of mitochondria (Y).**

Y (%) = **F** × **Susp** (volume of the mitochondrial suspension, L^{-1}) ÷ **m** (muscle mass used in the preparetion of mitochondria, kg) × **100.**

APPENDIX IV. RESULTS

Incremental exercise tests in LTx and MC (1)

Subjects	$\dot{V}O_2$peak	Pred.	$\dot{V}O_2$/pred. %	HRpeak	Pred.	HR/pred. %	$\dot{V}$Epeak (L min^{-1})	MVV	$\dot{V}_E$/MVV
LTx1	16	31.57	51	118	176	67	81	73	111
LTx2	14.3	28.02	51	121	178	68	39	48	82
LTx3	16.1	24.94	65	132	182	73	39	83	47
LTx4	21.6	32.34	67	159	188	85	80	122	66
LTx5	15.9	26.56	60	137	193	71	81	113	71
LTx6	23.8	48.41	49	139	191	73	64	119	54
LTx7	23.1	48.13	48	153	194	79	65	90	73
MC1	32.10	28.82	111	166	176	95	105	133	79
MC2	33.42	26.72	125	189	180	105	90	124	72
MC3	44.33	43.17	102	183	193	95	148	140	105
MC4	31.78	28.70	110	159	182	87	95	115	82
MC5	36.42	30.17	120	164	187	88	129	153	84
MC6	33.11	31.72	104	185	192	96	86	132	65
MC7	47.36	45.38	104	195	192	101	177	152	116

$\dot{V}O_2$peak= peak oxygen uptake; HR = heart rats. $\dot{V}$Epeak = minute ventilation at peak exercise; MVV =. predicted maximum voluntary ventilation (FEV_1 X 35). Pred. = predicted value. % = measured value / predicted value %.

Incremental exercise tests in LTx and MC (2)

Subjects	Exercise duration (min)	Workrate Peak (watts)	Rest SaO_2 (%)	Lowest SaO_2 (%)	De-SaO_2 (%)	Reasons for Termination exercise
LTx1	7	114	98	95	-3.06	AL&SOB.
LTx2	5	81	97	94	-3.09	AL
LTx3	3	49	99	98	-1.01	AL
LTx4	5	81	97	97	0	AL
LTx5	5	81	97	96	-1.03	AL
LTx6	7	114	98	99	1.02	AL & SOB
LTx7	6	98	99	98	-1.01	AL
MC1	9	225	97.4	96.75	-0.65	
MC2	6	150	94.9	94.9	0	
MC3	13	325	96.05	93.7	-2.35	
MC4	8.	200	95.1	95.4	+0.3	
MC5	8	200	97.45	97.45	0	
MC6	7	175	97	96.45	-0.55	
MC7	14	350	96.85	96.7	-0.15	

SaO_2 = blood oxygen saturation. De-SaO_2 = blood oxygen desaturation. LT = lactate threshold. AL = aching leg. SOB = short of breath. LF = leg fatigue.

Incremental exercise tests in RT and UT

Subjects	$\dot{V}O_2$peak (ml min^{-1}kg^{-1})	HRpeak (bpm)	Exercise Duration (min)	Workrate Peak (watts)	Subjects	$\dot{V}O_2$peak (ml min^{-1}kg^{-1})	HRpeak (bpm)	Exercise Duration (min)	Workrate Peak (watts)
UT1	44.82	179	14	350	RT1	39.26	189	14	350
UT2	50.51	200	12	300	RT2	28.95	176	10	250
UT3	44.30	183	13	325	RT3	45.27	189	13	325
UT4	33.18	186	12	300	RT4	52.74	189	14	350
UT5	47.32	195	14	350	RT5	58.2	186	16	400
UT6	47.61	192	14	350	RT6	47.04	171	13	325
UT7	43.99	200	12	300	RT7	38.05	190	13	325
UT8	43.58	182	13	325	RT8	47.47	188	15	375

Peak muscle torque (Nm) at different angular velocities ($^{\circ}$ s^{-1}) in RT and UT

Subjects	Isometric 0°	0	60	120	180	240	300	360	Total work (W)
RT1	216.3	207.0	230.4	185.1	161.6	137.4	125.7	109.2	5760
RT2	229.7	217.0	233.6	198.5	167.6	143.9	126.7	103.9	5739
RT3	194.6	180.0	215.3	185.0	151.3	134.8	118.2	104.0	5185
RT4	218.6	205.0	199.3	167.5	147.5	137.9	126.5	110.8	6025
RT5	231.6	207.0	192.7	163.1	129.8	113.4	99.8	102.1	5806
RT6	187.5	179.0	178.4	158.1	130.7	113.8	101.8	98.9	5494
RT7	244.5	237	240.7	219.8	194.3	175.5	160.1	148.6	7932
RT8	263.2	254.0	244.6	213.2	189.4	160.1	157.8	134.1	6519
UT1	213.2	201	181.3	169.1	149.4	120.3	109.4	93.8	4905
UT2	186.6	167	171.9	156.5	129.5	120.3	114	93.4	4975
UT3	213.2	194	228.5	176.7	148.2	135.2	131.3	116.1	5590
UT4	180.1	172	198.1	159.9	137.9	115	112	93.6	4784
UT5	253	245	208.4	165.2	130.7	111.9	92.5	100.3	4872
UT6	211.4	191	175.9	150.9	134.5	109.6	106.7	92.2	4801
UT7	203.3	193	183.6	138.6	117.3	94.1	88.3	77.7	5124
UT8	220.8	213	195.9	161	129.5	120.4	100.1	80.5	4883

Peak torque, fatigue index and plasma lactate concentration in Cybex dynamometer test in RT and UT.

Subjects	Cybex Peak-Tordue (Nm)	Cybex FI (%)	PL Rest	PL 25-E.	PL 50-E	PL R1	PL R2	PL R5	PL R10	PL R30
RT1	123	51	2.44	3.94	4.63	6.78	7.58	7.76	6.62	3.41
RT2	109.3	54	1.77	1.54	2.06	2.99	4.30	5.94	5.33	2.48
RT3	123	41	1.63	3.87	4.44	4.44	4.44	4.41	4.24	1.40
RT4	129.9	53	1.33	3.49	4.91	8.64	10.40	6.69	7.98	2.54
RT5	97.6	56	-	-	-	-	-	-	-	-
RT6	97.6	13	1.31	2.21	2.66	2.75	3.03	3.49	3.93	1.47
RT7	154.7	49	1.25	2.08	2.83	4.23	5.78	8.35	5.19	2.73
RT8	113.7	22	1.73	2.61	3.94	5.19	5.19	6.10	4.37	1.61
UT1	132.8	39	1.27	2.25	3.62	5.83	5.32	4.54	3.57	1.53
UT2	121.1	58	0.84	3.18	3.91	8.49	8.63	7.12	8.08	2.16
UT3	128.9	42	1.23	2.18	3.65	5.15	5.79	5.10	4.67	2.65
UT4	106.4	35	1.53	2.47	5.62	6.93	7.72	7.57	5.05	2.22
UT5	105.4	42	0.41	2.57	4.27	5.68	6.64	6.33	3.18	0.87
UT6	118.6	57	1.10	2.79	6.01	8.78	9.40	11.93	11.18	3.86
UT7	121.1	47	1.26	1.34	2.71	3.02	3.92	3.49	3.28	1.37
UT8	127.4	56	0.91	2.07	3.91	6.47	7.22	4.98	2.68	2.00

FI = fatigue index. PL = Plasma lactate concentration (mmol L^{-1}). E = leg exercise. R = recovery.

Plasma lactate concentration (mmol L^{-1}) in LTx and MC during incremental exercise.

Time	R	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14
LTx1	0.7	0.9	1.1	1.6	2.1	2.9	3.8	5.4							
LTx2	1.3	1.4	1.7	2.7	3.8	5.5									
LTx3	1.6	1.9	2.7	3.9											
LTx4	1.3	1.5	2.1	3.1	4.3	6.4									
LTx5	2.3	2.7	3.6	5	5.6	6.1									
LTx6	0.8	1	1.5	2.2	3.4	4.4	5.8	8.4							
LTx7	2.4	2.4	2.8	3.4	4.5	5.6	8.4								
MC1	1.2	1.3	1.3	1.6	2.0	2.8	4.2	5.4	7.7	8.7					
MC2	0.9	1.2	1.7	2.6	3.7	4.6	7.9								
MC3	0.8	1.0	1.0	1.0	1.3	1.6	1.9	2.2	2.9	3.7	5.5	6.9	9.3	11	
MC4	0.9	0.9	1.0	1.3	1.8	2.4	3.9	6.0	8.8						
MC5	1.2	2.0	2.1	2.7	3.9	4.7	6.2	7.7							
MC6	0.9	0.9	1.6	2.2	2.9	3.6	4.3	6.6	9.2						
MC7	0.8	0.8	0.9	1.1	1.2	1.5	1.9	2.5	3.1	4.5	5.9	7.9	7.9	9.6	12.5

Lactate threshold (LT) during incremental exercise test in LTx and MC

Subjects	Workrate (watts)	$\dot{V}O_2peak$ (ml kg^{-1} min^{-1})
LTx1	33.11	6.9
LTx2	28.18	7.8
LTx3	39.81	10.0
LTx4	23.82	8.3
LTx5	23.39	10.1
LTx6	25.7	5.1
LTx7	41.21	10.1
MC1	81.47	17.0
MC2	31.62	11.2
MC3	104.7	20.0
MC4	56.23	15.0
MC5	70.79	15.9
MC6	83.8	17.5
MC7	104.7	20.0

Plasma lactate concentration (mmol L^{-1}) in RT and untrained UT.

	R	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E 11	E 12	E 13	E 14	E 15	E16
RT1	1.1	1.1	1.2	1.4	1.5	2.1	2.1	2.7	3.1	3.4	5.2	6.6	8.2	11	15		
RT2	1.3	1.4	2.1	2.2	3.1	4.1	5.1	6.2	7.4	10	11.4						
RT3	1.1	1.8	2.4	2.6	2.9	3.0	3.6	4.7	5.6	6.6	7.7	11	14.4	17			
RT4	0.5	0.8	1.4	1.8	2.2	3.2	3.9	4.9	6.0	6.4	8.6	8.7	11.3	13	15		
RT5	0.8	1.1	1.1	1.5	1.6	1.7	1.7	1.7	1.8	1.9	1.9	2.9	2.8	3.3	4.4	5	7.0
RT6	1.5	1.6	1.3	1.6	2.4	2.4	3.6	3.5	4.5	5.8	7.4	9.9	11.4	29			
RT7	1.0	1.3	1.5	1.8	2.6	3.1	3.6	5.1	6.2	9.1	10.4						
RT8	0.3	2.1	2.2	2.6	3.0	3.1	3.4	3.5	4.0	4.5	5.6	6.9	8.4	11	14	18	
UT1	0.8	0.9	0.9	1.0	1.2	1.4	1.4	2.0	2.3	3.0	4.2	6.1	8.2	11	12		
UT2	0.8	0.9	1.0	1.2	1.7	3.1	4.4	6.0	8.0	9.4	10.8	16					
UT3	0.8	1.0	1.0	1.0	1.3	1.6	1.9	2.2	2.9	3.7	5.5	6.9	9.3	11			
UT4	0.7	0.8	1.0	1.9	2.7	4.1	3.1	4.2	5.3	6.5	8.5	12	13.9				
UT5	0.8	0.8	0.9	1.1	1.2	1.5	1.9	2.5	3.1	4.5	5.9	7.7	7.9	13	17		
UT6	1.0	0.9	1.2	1.3	1.4	1.8	2.7	3.2	3.7	5.1	5.5	7.7	9.6	11			
UT7	1.2	1.5	1.9	1.8	2.7	3.2	3.9	4.5	6.4	8.4	10.4	13	16.				
UT8	0.6	0.6	0.8	0.9	1.1	1.4	1.9	2.5	3.5	4.7	6.2	8.1	11.4	15			

Lactate threshold (LT) in RT and UT.

Subjects	Workrate (watts)	$\dot{V}O_2peak$ (ml^{-1} kg^{-1} min)
RT1	122.8	19.28
RT2	75.6	10.19
RT3	199.04	25.97
RT4	72.9	11.41
RT5	222.88	35.76
RT6	97.5	16.59
RT7	123.99	13.27
RT8	199.91	28.34
UT1	122.9	18.79
UT2	75.6	24.83
UT3	104.7	20.0
UT4	49.15	10.54
UT5	104.7	20.0
UT6	122.8	16.66
UT7	97	23.36
UT8	133.99	18.37

Skeletal muscle fibre types in LTx and MC

Subjects	Total Fibers No.	Type I No.	%	Type II No.	%
LTx1	288	91	31.60	197	68.40
LTx2	245	85	34.69	160	65.31
LTx3	274	43	15.69	231	84.31
LTx4	177	89	50.28	88	49.72
LTx5	348	53	15.23	295	84.77
LTx6	250	8	3.20	242	96.80
LTx7	120	28	23.33	92	76.67
MC1	225	129	57.33	96	42.67
MC2	178	108	60.67	70	39.33
MC3	376	236	62.77	140	37.23
MC4	304	153	50.33	151	49.67
MC5	188	113	60.11	75	35.89
MC6	258	123	45.21	135	54.79
MC7	404	228	56.44	176	43.56

No. = numbres of muscle fibres. % = percentage for one type fibres / total fibres.

Skeletal muscle fibre types in RT and UT subjects.

Subjects	Total Fibers No.	Type I No.	%	Type II No.	%	Type IIa No.	%	Type IIb No.	%
RT1	133	44	33.08	89	66.92	71	53.38	18	13.54
RT2	193	94	48.70	99	51.30	54	27.98	45	23.32
RT3	222	132	59.46	90	40.54	87	39.19	3	1.35
RT4	161	70	43.48	91	56.52	72	44.72	19	11.80
RT5	214	144	67.29	70	32.71	70	32.71	0	0.00
RT6	165	107	64.85	58	35.15	53	32.12	5	3.03
RT7	224	108	48.21	116	51.79	98	43.75	18	8.04
RT8	128	46	35.94	82	64.06	68	53.13	14	10.93
UT1	308	147	47.73	161	52.27	121	39.29	40	12.98
UT2	212	77	36.32	135	63.68	83	39.15	52	24.53
UT3	376	236	62.77	140	37.23	131	34.84	9	2.39
UT4	193	94	48.70	99	51.30	54	27.98	45	23.32
UT5	404	228	56.44	176	43.56	176	43.56	0	0.00
UT6	226	134	59.29	92	40.71	81	35.84	11	4.87
UT7	112	52	46.43	60	53.57	45	40.18	15	13.39
UT8	399	256	64.16	143	35.84	134	33.58	9	2.26

No. = numbers of muscle fibres. % = percentage for one type fibres / total fibres.

Resting muscle metabolites measured by HPLC (H) and by enzymatic analyses (E) in LTx and MC

Subjects	ATP(H)	ATP(E)	ADP	AMP	IMP	PCr	Cr	Lactate	Glycogen
LTX1	27.52	20.73	3.84	0.13	0.31	118.47	51.74	17.22	291.54
LTX2	19.88	22.83	2.76	0.07	0.17	106.31	74.19	15.84	394.23
LTx3	18.41	20.99	2.32	0.05	0.28	92.54	37.16	14.74	711.34
LTx4	18.70	20.37	2.76	0.06	0.15	97.02	56.45	12.83	320.61
LTx5	21.51	21.94	2.82	0.14	0.50	98.32	54.36	14.48	281.71
LTx6	22.32	24.64	2.74	0.17	0.23	77.77	47.17	19.99	510.90
LTx7	21.50	25.72	2.43	0.20	0.23	87.27	64.09	18.74	718.33
MC1	33.33	29.26	2.49	0.47	0.07	98.90	58.49	4.55	392.43
MC2	24.74	23.82	2.34	0.37	0.10	79.18	48.72	10.82	427.08
MC3	26.54	27.35	2.06	0.11	0.04	93.94	34.54	9.42	578.61
MC4	26.05	24.82	2.41	0.14	0.02	96.28	39.34	6.70	421.07
MC5	23.50	23.13	2.15	0.07	0.06	92.71	55.17	8.51	479.33
MC6	24.00	22.13	2.29	0.29	0.03	100.52	57.32	10.87	590.66
MC7	23.63	25.87	3.16	0.20	0.04	90.76	34.59	7.93	344.17

Values are expressed as mmol kg^{-1} dw. ATP(H), ADP, AMP and IMP measured by HPLC. ATP(E), PCr, Cr, lactate and glycogen measured enzymaticly.

Muscle metabolites measured by HPLC (H) analyses in RT and UT subjects

Subjects	ATP(H) Rest	Fatifue	ADP Rest	F)atigue	AMP Rest	F)atigue	IMP Rest	Fatigue
RT1	31.20	23.16	3.05	3.30	0.07	0.09	0.15	5.70
RT2	30.10	24.21	2.80	2.63	0.09	0.12	0.03	0.86
RT3	23.08	23.56	2.57	2.91	0.06	0.06	0.15	0.75
RT4	28.12	25.27	2.89	2.53	0.07	0.10	0.21	1.07
RT5	24.32	26.81	3.75	4.77	0.19	0.26	0.05	0.79
RT6	27.43	20.48	3.99	2.46	0.08	0.19	0.04	0.90
RT7	17.43	13.97	1.99	3.06	0.03	0.12	0.08	5.21
RT8	25.71	22.79	4.57	4.53	0.29	0.24	0.10	1.45
UT1	24.22	27.03	3.16	5.47	0.16	0.22	0.09	1.29
UT2	27.71	20.08	2.30	2.30	0.07	0.08	0.07	2.30
UT3	24.74	21.81	2.06	2.44	0.11	0.09	0.10	2.54
UT4	29.16	27.42	2.74	2.92	0.11	0.11	0.11	2.92
UT5	23.63	22.08	2.15	3.95	0.20	0.20	0.04	4.15
UT6	22.80	17.15	3.19	1.07	0.22	0.22	0.09	1.92
UT7	22.31	18.42	3.34	2.87	0.11	0.14	0.03	8.79
UT8	23.12	17.31	2.22	2.14	0.09	0.11	0.09	2.14

Values are expressed as mmol kg-1 dw

Muscle metabolites measured enzymatically in RT and UT subjects

Subjects	ATP(E) Rest	Fatigue	PCr. Rest	Cr. Rest	TCr. Rest	PCr. Fatigue	Cr. Fatigue	TCr. Fatigue	Glycogen Rest	Fatigue	Lactate Rest	Fatigue
RT1	28.51	20.9	108.481	49.66	158.14	36.27	121.87	158.14	554.82	483.19	10.64	103 13
RT2	26.88	20.69	109.36	38.74	148.10	43.02	105.08	148.10	562.96	387.44	6.64	59.57
RT3	26.77	23.96	101.314	34.62	135.93	30.69	105.24	135.93	513.39	370.74	12.19	59.11
RT4	27.16	23.3	104.397	42.03	146.43	54.7	91.73	146 43	336.99	314.71	11.26	67.87
RT5	30.07	29 19	118.655	43.27	161.93	48.15	113.77	161.92	403.68	311 06	7 98	64.36
RT6	24.64	13.21	101.18	63.45	164.63	53.35	111.28	164.63	520 47	407.94	9.63	115.64
RT7	22.37	12.15	92.79	50.22	143.01	36.02	106.99	143.01	464.56	263.78	9.05	92.58
RT8	26.69	19.09	105.64	36.22	141.86	38.41	103.45	141.86	470.57	323.85	13.20	118.42
UT1	26.31	27.12	91.72	32.51	124.23	22.31	101.92	124.23	446.13	309.06	10.99	82.74
UT2	29.42	19.49	105.41	43.97	149.37	37.18	112.20	149.37	450.76	336.32	5.02	92.60
UT3	27.21	21.72	93.94	34.54	128.48	46.39	82.09	128.48	578.61	550.37	10.82	72.53
UT4	25.35	21.99	99.23	53.00	152.23	41.29	110.94	152.23	445.10	350.92	7.13	76.13
UT5	25.87	20.52	90.76	34.59	125.35	47.61	77.74	125.35	344.17	250.62	7.93	71.25
UT6	22.29	12.50	90.14	45.43	135.57	27.5	108.07	135.57	433.46	371.18	9.82	128.04
UT7	22.35	12.83	83.22	44.35	127.57	24.61	102.96	127.57	644.63	359.22	6.53	142.44
UT8	26.61	19.90	91.84	47.86	139.70	39.33	100.37	139.70	409.01	321.21	6.07	66.74

Values are expressed as mmol kg^{-1} dw. ATP(E)= ATP measured enzymatically.

Activity of muscle enzymes in LTx and MC (mmol $min^{-1}kg^{-1}$ w. w)

Subjects	CS	KGDH	HAD	PFK	PK	HK	PHOSPH
LTx1	5 48	0.24	2.49	42.82	300.33	1.34	20.21
LTx2	6.98	0.15	2 54	30 70	130.32	0.94	14.01
LTx3	15.06	0.46	2.47	40.59	245.52	1.43	20.81
LTx4	7.20	0.32	3.81	34.115	252.12	1.99	13.09
LTx5	10.5	0.65	2.145	44.85	317.32	2.84	18.03
LTx6	11.14	0.31	2.06	37.7531	274.36	1.62	18.33
LTx7	14.99	0.63	3.13	22.785	119.79	1.45	6.86
MC1	21.93	1.28	5.83	22.02	299.70	1.35	12.08
MC2	12.49	0.79	4.14	25.23	217.74	1.48	14.53
MC3	19.53	1.05	4.23	31.19	251.19	1.49	14.09
MC4	14.79	0.83	5.62	27.49	218.34	1.42	12.60
MC5	18.13	0.85	4.2	27.58	302.69	1.39	14.60
MC6	20.86	0.93	4.02	35.34	273.45	2.06	19.76
MC7	22.94	1.33	4.89	27.48	272.25	2.34	12.73

Activity of muscle enzymes in RT and UT (mmol $min^{-1}g^{-1}$ muscle protein)

Subjects	CS	GDH	KGDH	HAD	PFK	PK	HK	PHOSPH
RT1	101.9	6.13	3.74	21.03	223.5	1587.0	14.25	117.6
RT2	84.9	5.29	0.99	19.57	223.2	1494.2	11.84	141.6
RT3	102.6	4.18	4.84	28.37	199.4	1437.0	13.91	113.4
RT4	84.3	6.13	4.25	22.28	238.0	1470.6	10.61	118.6
RT5	137.8	6.39	8.10	28.58	164.3	1890.7	12.50	78.6
RT6	101.5	5.58	4.94	23.41	143.0	1525.7	16.45	70.5
RT7	92.3	5.76	4.80	17.23	219.5	942.4	12.11	89.1
RT8	162.9	9.76						
UT1	114.5	5.22	7.37	30.81	174.9	765.9	6.68	83.0
UT2	108.8	5.05	3.43	24.77	161.2	1083.4	10.60	100.5
UT3	108.4	4.89	4.83	20.89	183.7	1315.2	10.68	75.7
UT4	88.0	4.67	2.41	22.55	172.3	861.2	9.50	95.6
UT5	128.7	7.88	3.72	30.54	171.6	1699.8	14.58	79.5
UT6	146.4	7.59	6.30	31.62	222.1	1677.6	14.94	102.7
UT7	136.0	6.57	4.89	24.59	211.1	1717.3	13.77	88.4
UT8	92.0	3.39			176.5	1124.9	10.05	100.1

MAPR in LTx and MC

	(mmol min^{-1} g^{-1} protein in mitochondrial suspension)					(mmol min^{-1} kg^{-1} muscle weight)				
Snbjects	PPKM	S + R	A-KG	PC-M	P + M	PPKM	S + R	A-KG	PC-M	P + M
LTx 1	0.25	0.27	0.20	0.20	0.18	2.11	2.27	1.73	1.72	1.56
LTx 2	0.25	0.27	0.11	0.09	0.19	3.25	3.54	1.49	1.25	2.51
LTx 3	0.80	0.44	0.38	0.24	0.35	8.79	4.79	4.11	2.64	3.87
LTx 4	0.69	0.57	0.42	0.24	0.28	7.72	6.38	4.66	2.64	3 17
LTx 5	0.25	0.18	0.12	0.10	0.20	2.06	1 45	0.94	0.81	1 59
LTx 6	0.19	0.19	0.09	0.15	0.10	1.45	1.47	0.66	1 13	0.76
LTx 7	0.40	0.30	0.18	0.13	0.36	5.56	4.24	2.52	1.75	4.99
MC 1	0.57	0.39	0.42	0.30	0.41	10.21	7.00	7.45	5.30	7.31
MC 2	0.58	0.32	0.39	0.29	0.34	11.32	6.18	7.71	5.64	6.74
MC 3	0.74	0.54	0.42	0.24	0.33	12.54	9.18	7.15	4.12	5.63
MC 4	0.61	0.30	0.34	0.32	0.41	10.22	4.96	5,70	5.26	6.76
MC 5	0.53	0.21	0.23	0.24	0.28	7.72	3.12	3.42	3.56	4.08
MC 6	0.64	0.45	0.61	0.37	0.37	9.01	6.32	8.68	5 24	5.23
MC 7	0.72	0.38	0.38	0.31	0.45	13.19	6.96	6.91	5.68	8.27

MAPR in RT and UT

	(mmol min^{-1} g^{-1} protein in mitochondrial suspension)					(mmol min^{-1} kg^{-1} muscle weight)				
Subjects	PPKM	S + R	A-KG	PC + M	P + M	PPKM	S + R	A-KG	PC + M	P + M
RT 1	0.43	0.39	0.24	0.16	0.24	8.19	7.43	4.58	3.13	4.52
RT 2	0.43	0.37	0.42	0.23	0.25	9.08	7.68	8.74	4.76	5.14
RT 3	0.88	0.48	0.66	0.40	0.40	10.53	5.77	7.91	4.78	4.76
RT 4	0.40	0.29	0.26	0.26	0.37	9.53	6.85	6.21	6.13	8.93
RT 5	0.82	0.38	0.37	0.20	0.48	13.53	6.31	6.14	3.25	7.98
RT 6	0.64	0.34	0.43	0.30	0.43	9.64	5.15	6.50	4.55	6.55
RT 8	0.55	0.36	0.37	0.29	0.42	10.69	7.04	7.20	5.72	8.07
UT 1	0.59	0.38	0.29	0.30	0.21	11.72	7.61	5.86	5.91	4.10
UT 2	0.43	0.33	0.33	0.19	0.33	8.50	6.64	6.56	3.83	6.51
UT 3	0.74	0.54	0.42	0.24	0.33	12.54	9.18	7.15	4.12	5.63
UT 4	0.62	0.41	0.52	0.30	0.45	10.81	7.13	8.96	5.21	7.75
UT 5	0.72	0.38	0.38	0.31	0.45	13.19	6.96	6.91	5.68	8.27
UT 6	0.49	0.32	0.36	0.26	0.32	10.37	6.78	7.67	5.53	6.84
UT 7	0.79	0.55	0.67	0.40	0.53	13.13	9.14	11.21	6.66	8.79
UT 8	0.32	0.13	0.23	0.13	0.34	6.60	2.61	4.59	2.66	6.96

MAPR in the presence of various substrates and different concentrations of cyclosporine A (CyA)

Subjstrates /rats	50 µmol of CyA ml^{-1}	25µmol of CyA ml^{-1}	1µmol of CyA ml^{-1}	0µmol of CyA ml^{-1}	Substrates/ rats	50 µmol of CyA ml^{-1}	25µmol of CyA ml^{-1}	1µmol of CyA ml^{-1}	0µmol of CyA ml^{-1}
P + M					S + R				
No. 1	0.23	0.23	0.25	0.28	No.1	0.19	0.21	0.24	0.28
No.2	0.44	0.44	0.44	0.43	No.2	0.19	0.2	0.23	0.26
No.3	0.31	0.34	0.36	0.39	No.3	0.19	0.21	0.28	0.31
No.4	0.53	0.52	0.53	0.64	No.4	0.4	0.4	0.47	0.6
No.5	0.27	0.35	0.43	0.57	No.5	0.16	0.16	0 17	0.18
No.6	0.72	0.7	0.9	0.9	No.6	0.25	0.23	0.29	0.33
No.7	0.53	0.57	0.75	0.79	No.7	0.11	0.14	0.17	0.18
No.8	0.34	0.43	0.44	0.56	No.8	0.17	0.16	0 17	0 2
PC + M					PPKM				
No.1	0.27	0.27	0.26	0.29	No.1	0.41	0.35	0.39	0.39
No.2	0.3	0.28	0.26	0.26	No.2	0.56	0.53	0.46	0.59
No.3	0.31	0.34	0.38	0.39	No.3	0.44	0.52	0.6	0.61
No 4	0.33	0.3	0.36	0.43	No.4	0.63	0.61	0.68	0.73
No.5	0.23	0.26	0.32	0.35	No.5	0.44	0.47	0.56	0.63
No.6	0.34	0.32	0.43	0.48	No.6	0.71	0.79	0.87	1
No.7	0.24	0.24	0.31	0.34	No.7	0.64	0.53	0.7	0.89
No.8	0.27	0.26	0.27	0.35	No.8	0.56	0.49	0.56	0.69

Values are expressed as mmol min^{-1} g^{-1} protein.

Activity of GDH, muscle and mitochondrial suspension protein in LTx and MC

Subjects	GDHim	GDHt	GDHim/GDHt	Mit. Sus. Pro. (mg ml^{-1})	Mit.Sus. Pro/mw (g kg^{-1})	Mit. Sus. Pro. in assay (ng ml^{-1})	Muscle Protein/mw (%)
LTx1	0.015	0.42	0.036	0.30	1.22	12.10	16.52
LTx2	0.014	0.52	0.027	0.36	2.29	14.36	11.05
LTx3	0.026	0.74	0.036	0.39	1.95	15.62	15.33
LTx4	0.019	0.52	0.036	0.40	2.37	16.10	16.81
LTx5	0.010	0.53	0.020	0.16	1.09	6.33	16.58
LTx6	0.028	0.51	0.054	0.41	2.79	16.54	11.71
LTx7	0.047	0.71	0.067	0.93	4.14	37.27	14.15
MC1	0.068	1.10	0.062	1.10	5.78	43.28	14 46
MC2	0.030	0.82	0.036	0.71	4.37	16.25	16.09
MC3	0.028	0.97	0.029	0.49	3.42	24.20	17.9
MC4	0.021	0.86	0.024	0.41	2.35	28.41	13.5
MC5	0.040	0.96	0.042	0.61	4.03	20.68	9 8
MC6	0.031	0.85	0.037	0.52	3.18	19.40	15.1
MC7	0.037	1.26	0.030	0.54	3.60	21.71	16.01

GDHim = activity of GDH of intact mitochondria (mmol.$min^{-1}L^{-1}$). GDHt = total GDH (mmol.$min^{-1}kg^{-1}$). Mit. Sus. Pro.= mitochondrial suspension protein concentration. Mw = muscle weight.

Activity of GDH, muscle and mitochondrial suspension protein in RT and UT

Subjects	GDHim	GDHt	GDHim/GDHt	Mit. sus. pro. (mg ml^{-1})	Mit.sus. pro/mw (g kg^{-1})	Mit. sus. pro. In assay (ng ml^{-1})	Muscle protein/mw (%)
RT1	0.02	1.10	0.020	0.38	4.90	16.00	17.9
RT2	0.03	0.84	0.034	0.70	5.27	28.14	15.84
RT3	0.06	0.81	0.073	0.87	4.82	34.80	19 3
RT4	0.03	1.19	0.028	0.67	3.34	28.00	19.4
RT5	0.03	1.01	0.030	0.50	3.32	19.90	15.8
RT6	0.04	1.05	0.036	0.50	3.20	21.79	18.82
RT7	-	-	-	-	-	-	18.75
RT8	0.07	1.29	0.051	0.98	8.19	39.33	13.19
UT1	0.024	0.92	0.026	0.53	3.49	21.10	17.56
UT2	0.037	1.01	0.036	0.73	4.28	29.02	19.96
UT3	0.028	0.97	0.029	0.49	3.42	19.40	19.24
UT4	0.013	0.89	0.015	0.26	2.20	10.38	18.67
UT5	0.037	1.26	0.030	0.54	3.61	21.71	16.01
UT6	0.042	0.96	0.043	0.92	4.21	36.91	12.62
UT7	0.049	0.95	0.051	0.85	4.96	34 12	14 52
UT8	0.012	0.64	0.019	0.39	2.99	15.56	18.82

GDHim = activity of GDH of intact mitochondria (mmol.$min^{-1}L^{-1}$). GDHt = total GDH (mmol.$min^{-1}kg^{-1}$). Mit. Sus. Pro.= mitochondrial suspension protein concentration. mw = muscle weight

APPENDIX V. COMPARING THE TWO SYSTEMS FOR EXERCISE TESTING USED IN CHAPTER 4.

The systems for exercise testing at The Alfred Hospital (AH) and Victoria University of Technology (VUT) were compared for 3 untrained subjects.

Results of increment exercise tests conducted in Alfred hospital and Victoria University of Technology

			VUT				AH			
Time (min)	Workmate (watts)	Sample	$\dot{V}O_2$ (L min^{-1})	$\dot{V}CO_2$ (L min^{-1})	$\dot{V}O_2$ (ml min^{-1} kg^{-1})	$\dot{V}E$ (L min^{-1})	$\dot{V}O_2$ (L min^{-1})	$\dot{V}CO_2$ (L min^{-1})	$\dot{V}O_2$ (ml $min^{-1}kg^{-1}$)	$\dot{V}E$ (L min^{-1})
Subject 1										
0	0	Rest	0.55	0.49	6.06	20.78	0.47	0.65	5.22	26.9
1	25	E1	0.97	1.13	10.68	43.36	0.77	0.91	8.55	31.87
2	50	E2	0.88	1.42	9.68	58.22	0.88	0.97	9.82	35.83
3	75	E3	1.04	1.25	11.51	50.87	1.09	1.16	12.07	43.83
4	100	E4	1.31	1.49	14.45	59.37	1.17	1.14	13.03	42.53
5	125	E5	1.49	1.57	16.49	59.24	1.54	1.49	17.08	55.67
6	150	E6	1.89	1.6	20.92	54.93	1.57	1.53	17.48	56.17
7	175	E7	2.21	2.14	24.39	70.52	1.87	1.81	20.8	65.23
8	200	E8	2.38	2.23	26.26	70.84	2.03	1.9	22.53	63.97
9	225	E9	2.88	3.1	31.81	93.97	2.26	2.11	25.07	64.37
10	250	E10	3.09	3.58	34.17	110.04	2.51	2.46	27.85	73.23
11	275	E11	3.3	4.16	36.43	144.1	2.73	2.9	30.37	88.47
12	300	E12	3.36	4.34	37.16	191.1	3.09	3.35	34.36	104.37
13	325	E13					3.52	3.98	39.07	144.43
14	350	E14					3.89	4.25	43.18	193.35
Subject 2										
0	0	Rest	0.41	0.35	5.16	13.33	0.37	0.28	4.63	10.5
1	25	E1	0.56	0.46	7.04	16.15	0.62	0.48	7.77	16.73
2	50	E2	0.84	0.65	10.6	20.16	0.65	0.5	8.21	16.17
3	75	E3	1.03	0.86	13.06	24.77	0.93	0.74	11.76	21.8
4	100	E4	1.3	1.14	16.43	31.17	1.13	0.94	14.26	26.8
5	125	E5	1.55	1.5	19.55	40.02	1.35	0.8	17.05	33.2
6	150	E6	1.84	1.92	23.18	51.27	1.51	0.148	19.07	39.43
7	175	E7	2.06	2.25	26.06	60.75	1.86	1.91	23.54	51.17
8	200	E8	2.46	2.72	31.11	75.48	2.12	2.22	26.73	61.2
9	225	E9	2.73	3.16	34.46	92.94	2.29	2.51	28.95	72.87
10	250	E10	3.01	3.68	37.95	114.24	2.84	3.15	35.82	92.9
11	275	E11	3.06	4.16	38.58	153.34	2.92	3.43	36.89	113.23
12	300	E12					3.41	4.17	42.99	156.7
Subject 3										
0	0	Rest	0.3	0.31	2.92	15.42	0.3	0.28	2.92	12.6
1	25	E1	0.63	0.48	6.18	17.55	0.68	0.51	6.68	17.8
2	50	E2	0.88	0.67	8.58	21.95	0.88	0.7	8.6	21.63
3	75	E3	1.08	0.86	10.61	26.62	1.09	0.89	10.71	25.9
4	100	E4	1.38	1.13	13.52	33.54	1.27	1.07	12.42	29.73
5	125	E5	1.6	1.4	15.69	38.73	1.54	0.88	15.08	35.23
6	150	E6	1.91	1.76	18.77	45.27	1.72	1.54	16.82	40.5
7	175	E7	2.25	2.2	22.01	55.63	2.01	1.91	19.67	49.83
8	200	E8	2.57	2.63	25.22	65.83	2.24	2.21	22.01	57.27
9	225	E9	2.91	3.07	28.52	76.97	2.46	2.46	24.1	63.37
10	250	E10	3.07	3.44	30.07	89.65	2.78	2.85	27.27	74.5
11	275	E11	3.56	4.38	34.94	124.28	3.1	3.31	30.43	87.6
12	300	E12					173	91.6	3.71	1.1
13	325	E13					177	100	3.97	1.09

APPENDIX V. COMPARING THE TWO SYSTEMS FOR EXERCISE TESTING USED IN CHAPTER 4.

The systems for exercise testing at The Alfred Hospital (AH) and Victoria University of Technology (VUT) were compared for 3 untrained subjects.

Results of increment exercise tests conducted in Alfred hospital and Victoria University of Technology

			VUT				AH			
Time (min)	Workmate (watts)	Sample	$\dot{V}O_2$ (L min^{-1})	$\dot{V}CO_2$ (L min^{-1})	$\dot{V}O_2$ (ml min^{-1} kg^{-1})	$\dot{V}E$ (L min^{-1})	$\dot{V}O_2$ (L min^{-1})	$\dot{V}CO_2$ (L min^{-1})	$\dot{V}O_2$ (ml min^{-1}kg^{-1})	$\dot{V}E$ (L min^{-1})
Subject 1										
0	0	Rest	0.55	0.49	6.06	20.78	0.47	0.65	5.22	26.9
1	25	E1	0.97	1.13	10.68	43.36	0.77	0.91	8.55	31 87
2	50	E2	0.88	1.42	9.68	58.22	0.88	0.97	9.82	35.83
3	75	E3	1.04	1.25	11.51	50.87	1.09	1.16	12.07	43 83
4	100	E4	1.31	1.49	14.45	59.37	1.17	1.14	13.03	42.53
5	125	E5	1.49	1.57	16.49	59.24	1.54	1.49	17.08	55.67
6	150	E6	1.89	1.6	20 92	54 93	1.57	1.53	17.48	56.17
7	175	E7	2.21	2.14	24.39	70.52	1.87	1.81	20.8	65 23
8	200	E8	2.38	2.23	26.26	70.84	2.03	1.9	22.53	63 97
9	225	E9	2.88	3.1	31.81	93.97	2.26	2.11	25.07	64.37
10	250	E10	3.09	3.58	34.17	110.04	2.51	2.46	27.85	73.23
11	275	E11	3.3	4.16	36.43	144.1	2.73	2.9	30.37	88 47
12	300	E12	3.36	4.34	37.16	191.1	3.09	3.35	34.36	104.37
13	325	E13					3.52	3.98	39.07	144 43
14	350	E14					3.89	4.25	43.18	193 35
Subject 2										
0	0	Rest	0.41	0.35	5.16	13.33	0.37	0.28	4.63	10.5
1	25	E1	0.56	0.46	7.04	16.15	0.62	0.48	7.77	16.73
2	50	E2	0.84	0.65	10.6	20.16	0.65	0.5	8.21	16.17
3	75	E3	1.03	0.86	13.06	24.77	0.93	0.74	11.76	21 8
4	100	E4	1.3	1.14	16.43	31.17	1.13	0.94	14.26	26.8
5	125	E5	1.55	1.5	19.55	40.02	1.35	0.8	17.05	33.2
6	150	E6	1.84	1.92	23.18	51.27	1.51	0.148	19.07	39.43
7	175	E7	2.06	2.25	26.06	60.75	1.86	1.91	23.54	51.17
8	200	E8	2.46	2.72	31.11	75.48	2.12	2.22	26.73	61.2
9	225	E9	2.73	3.16	34.46	92.94	2.29	2.51	28.95	72.87
10	250	E10	3.01	3.68	37.95	114.24	2.84	3.15	35.82	92.9
11	275	E11	3.06	4.16	38.58	153.34	2.92	3.43	36.89	113 23
12	300	E12					3.41	4.17	42.99	156.7
Subject 3										
0	0	Rest	0.3	0.31	2.92	15.42	0.3	0.28	2.92	12.6
1	25	E1	0.63	0.48	6.18	17.55	0.68	0.51	6.68	17.8
2	50	E2	0.88	0.67	8.58	21.95	0.88	0.7	8.6	21.63
3	75	E3	1.08	0.86	10.61	26.62	1.09	0.89	10.71	25.9
4	100	E4	1.38	1.13	13.52	33.54	1.27	1.07	12.42	29.73
5	125	E5	1.6	1.4	15.69	38.73	1.54	0.88	15.08	35.23
6	150	E6	1.91	1.76	18.77	45.27	1.72	1.54	16.82	40.5
7	175	E7	2.25	2.2	22.01	55.63	2.01	1.91	19.67	49.83
8	200	E8	2.57	2.63	25.22	65.83	2.24	2.21	22.01	57.27
9	225	E9	2.91	3.07	28.52	76.97	2.46	2.46	24.1	63.37
10	250	E10	3.07	3.44	30.07	89.65	2.78	2.85	27.27	74.5
11	275	E11	3.56	4.38	34.94	124.28	3.1	3.31	30.43	87.6
12	300	E12					173	91.6	3.71	1.1
13	325	E13					177	100	3.97	1.09